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Thyroid Autoimmune Disease

Terry F. Davies, guest editor

From the Page and William Black Post-Graduate School of Medicine of the
Mount Sinai School of Medicine (CUNY) in New York and the
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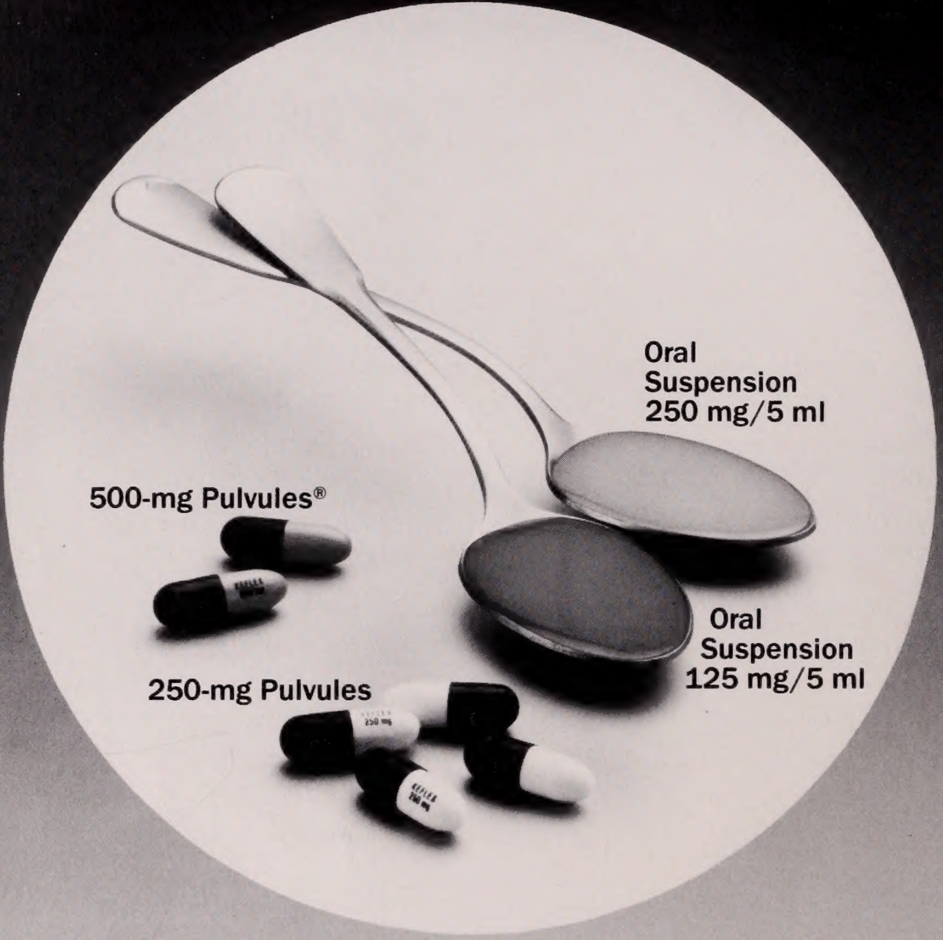
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Thyroid Autoimmune

Disease

THE MOUNT SINAI JOURNAL OF MEDICINE
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Thyroid Autoimmune Disease Introduction

Autoimmune thyroid disease is one of the commonest human afflictions; it affects at least 2% of the U.S. population. It may result in thyroid overactivity (Graves' disease) or thyroid failure (Hashimoto's disease). In addition, the realization in the last five to ten years that diabetes mellitus may also be an autoimmune phenomenon has made the search for an understanding of the disease mechanisms in autoimmune endocrine dysfunction even more imperative. Because thyroid disease is so common, and because people with the disease tend to survive for many years, it is an ideal model for the study of such human immunological disease mechanisms. Although thyroid autoantibodies were first discovered in 1956, we still do not understand the importance or the identity of many of them.

This issue of *The Mount Sinai Journal of Medicine* brings together contributions from personal friends who lead international groups attacking the basic problems of thyroid autoimmunity. Major advances in human genetics and the molecular biology of inherited diseases are beginning to allow us to closely examine autoimmune endocrine dysfunction, using these new approaches. I believe strongly that these new technological advances will provide a clearer understanding of the human autoimmune thyroid diseases.

The reports presented here are the culmination of a symposium held at the Mount Sinai School of Medicine in conjunction with the sixtieth annual meeting of the American Thyroid Association in New York City in September 1984 with Drs. Rose and Volpe as chairmen.

I want to take this opportunity to thank my colleagues for submitting manuscripts at a time when many demands are made upon them. They are each shining examples to the younger scientific community at a time when we are often bereft of suitable role models for our young physicians and basic scientists.

TERRY F. DAVIES
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Thyroid Autoimmune Disease

Part 1:

Genetic Predisposition

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Genetic Predisposition to Thyroid Autoimmune Disease: Introduction

Noel R. Rose, M.D., Ph.D., and C. Lynne Burek, Ph.D.

The appearance of multiple cases of autoimmune disease in members of the same family has been taken as evidence for a genetic predisposition to autoimmunity. Sometimes related individuals have the same disease; more often they have several different autoimmune diseases. Generally, these families have a heightened incidence of either the systemic autoimmune diseases, such as lupus erythematosus and rheumatoid arthritis, or the organ-specific autoimmune diseases, such as chronic thyroiditis, Graves' disease, insulin-dependent diabetes mellitus, and related autoimmune endocrinopathies; only a few have both.

In this discussion, we concentrate on autoimmune thyroid disease, in an effort to delineate how genetics may influence the development of autoimmunity and to speculate about the types of genetic factors that may be involved in disease production. Three models of autoimmune thyroiditis are available for genetic studies. First, human chronic thyroiditis is a classic example of organ-specific autoimmunity. Second, many of the essential features of the disease can be reproduced in experimental animals by immunization with the principal thyroid antigen, thyroglobulin. Third, spontaneous forms of thyroiditis occur in certain genetically predisposed animals, such as obese-strain chickens, Buffalo rats, and the Argonne colony of beagle dogs.

Spontaneous Thyroiditis in Animals. Important lessons about the genetic control of thyroid autoimmunity have been learned from studies of spontaneous thyroiditis in the obese-strain chicken (1). In this closed flock, originally developed by R. K. Cole of Cornell University in 1966 (2), the incidence of autoimmune thyroiditis is now over 90%. These animals develop autoantibodies to thyroglobulin and to thyroid epithelial cells, simulating those antibodies found in humans. The lesions in the thyroid gland closely resemble those of human Hashimoto's disease, and the chickens themselves show signs of profound hypothyroidism. The disease is clearly genetic in origin; no infectious agent has ever been found. In order to trace the genetic basis of this autoimmune disease, the noninbred obese strain was studied for a variety of genetic markers. The B blood group was closely associated with the development of thyroiditis (3).

Cell transfer studies have shown that the genetic defect is not in the thyroid gland or in the B cells (even though both B cells and the thyroid gland are essential for the development of antibody), but in the thymus-derived lymphocytes (4). The B blood group of the chicken is the major histocompatibility complex analogous to human HLA or murine H-2; therefore, it is logical to propose that the B-associated susceptibility to thyroiditis is based on an immunoregulatory gene.

A second genetic defect was discovered in the most susceptible of the obese-strain chickens. The thymus of these chickens matures abnormally. The normal sequence of events in chickens appears to be that the predominant population of lymphocytes leaving the thymus during embryonic life is of the suppressor T-cell type. Inducer/helper T cells probably migrate from the thymus somewhat later and are functionally the minority of cells in the periphery. The situation may be reversed in obese-strain birds; that is, the helper/inducer T cells may be the first to leave the thymus. This proliferation of helper/inducer cells

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may be maintained at a low level by contact with low levels of thyroglobulin during later life.

A third abnormality has been found in the thyroid gland of the obese-strain chicken. The first evidence for this abnormality was discovered by Wick, Sundick, and Albini (5) in the form of increased uptake of radioactive iodide but decreased production of T3 and T4.

Each of these three genetic defects in the obese-strain chicken sorts independently. The birds that are most prone to the development of severe autoimmune disease inherit all three defects: the B blood group associated locus for susceptibility; the abnormality in thymic maturation; and the intrinsic abnormality in the thyroid gland. Each of these three defects creates a different functional abnormality. The immunoregulatory gene probably determines response to one or a small number of antigenic determinants on the thyroglobulin molecule. The defect in thymic maturation predisposes to the development of a number of autoimmune reactions. The thyroid abnormality affects the function of the thyroid gland. One can understand, therefore, why the obese-strain chicken may develop a number of autoantibodies; nevertheless, the major autoantibody response is directed to thyroglobulin and the only significant disease is thyroiditis.

Experimentally Induced Thyroiditis. Most of the investigations of experimentally induced thyroiditis have been in the mouse; a larger number of inbred strains is available. Early investigations showed great variation in the response of various strains of mice to a standardized stimulus of murine thyroglobulin plus a suitable adjuvant. Genetic analysis of the strains showed that the response was predictable on the basis of their H-2 genotype (6). Certain alleles, such as H-2k and H-2s, are consistently associated with vigorous responses to thyroglobulin, as evidenced by a prompt production of thyroglobulin autoantibody and the early appearance of severe lesions in the thyroid gland. Other H-2 genotypes, such as H-2b or H-2d, are characterized by a later production of autoantibodies and mild or insignificant lesions.

Further genetic analyses were carried out using intra-H-2 recombinants. The major gene controlling responsiveness to thyroglobulin is located in the I-A subregion (7). This region corresponds to the DR region of human HLA. Further investigation showed that a second gene within H-2 influences the development of experimental thyroiditis in mice. This gene was localized at the D region of H-2. Its major effect was not on antibody production, but on the severity of thyroid infiltration.

In order to explain these results, we assume that there are two levels of genetic control. The first involves induction of the immune response and is encoded primarily at the I-A region. The second level of genetic control determines development of the effector response, as assessed by pathological lesions. This genetic control is localized in the D region. The K region also affects the severity of thyroiditis lesions.

Human Autoimmune Dysfunction. It is now obvious that the development of human autoimmune disease is determined to a great extent by polygenic inheritance (8, 9). The most commonly identified genetic factor is associated with the major histocompatibility complex (10). There are several ways in which HLA might play a role in autoimmune disease. The first, and most likely, is that HLA incorporates an immunoregulatory gene similar to the murine Ir genes. Individuals who inherit certain HLA haplotypes are probably better responders to particular antigenic determinants of self-antigens. However, as the experiments with murine thyroiditis have shown, the major histocompatibility complex can have several additional effects. Genetic susceptibility to the initiation of autoimmune response may differ from control of the effector arm of the immune response, which is responsible for the actual production of disease. Consequently the occurrence of autoantibodies is much more common than the development of lesions.

Although particular HLA types are significantly associated with autoimmune diseases, these associations are generally relatively weak (11).

One possible explanation is that the actual gene responsible for increased susceptibility is associated with the HLA determinant by linkage disequilibrium. Therefore, the HLA determinant serves only as the marker for the nearby disease-susceptibility gene. Thus far, no such disease-susceptibility gene has been identified, either in experimental animals or in human populations.

A second possibility is a transposition in the HLA gene itself. To find such an abnormality, it is necessary to compare restriction-fragment maps of DNA from diseased and normal individuals. It may be that analysis of restriction fragment length will demonstrate an abnormal sequence in those individuals who actually develop disease.

A third possibility is suggested by the accompanying papers as well as by our own experimental evidence. This evidence indicates that the development of autoimmune thyroid disease represents the conjunction of several independent genetic traits, as Knight and Knight suggest for murine lupus. In the case of thyroiditis, these events include the inheritance of one or more immunoregulatory genes associated with the histocompatibility locus discussed in the article by Farid et al, an abnormality in maturation of the thymus, and an increase in the major histocompatibility complex class II gene expression of the thyroid gland described by Davies and Piccinini. Individuals who inherit all three of these defects are the ones most likely to develop autoimmune disease. If only two defects are inherited, the frequency of disease might be considerably less. However, the intervention of developmental and environmental factors may shift the balance toward the development of disease. Such factors as sex hormones, thyroid infection, or even stress, may be additional contributors. In this respect, it is important to point out that full-blown thyroiditis can be induced even in genetically resistant mice and rats if there is a sufficiently strong antigenic stimulus. In the case of experimental thyroiditis, that stimulus is provided by thyroglobulin combined with Freund's adjuvant.

The concept just presented does not assume that there are disease-susceptibility genes as such. It is probably the conjunction of several abnormalities that is responsible for the spontaneous development of disease in some, but not all, individuals with a particular HLA haplotype (12). In families predisposed to the development of autoimmunity, different autoimmune diseases can develop in various members of the same family, as Roman describes in her discussion of family studies. This difference in autoimmune diseases is due in part to the occurrence of various target organ abnormalities in members of the family. Age and sex come into play because of their role in altering thymus function.

Recent investigations have established that a predisposition to the development of autoimmunity is genetically determined. It is quite likely that the delineation of these genetic factors will permit the identification of individuals at greatest risk of development of autoimmune disease.

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The Major Histocompatibility Complex and Autoimmune Thyroid Disease

Nadir R. Farid, MBBS, MRCP(UK), FRCP(C), FACP, Valeria Stenszky, Ph.D., M.D.,
Csaba Balazs, Ph.D., M.D., and John C. Bear, Ph.D.

The year 1956 was a significant one for both endocrinology and immunology. Roitt and Doniach described antithyroglobulin antibodies in the sera of patients with goitrous Hashimoto's thyroiditis (1), Adams and Purves postulated an abnormal thyroid stimulator in the serum of patients with active Graves' disease (2), and Rose and Witebsky created an animal model of autoimmune thyroiditis by immunizing rabbits with autologous thyroglobulin (3). In immunology, Jean Dausset described agglutination of white cells by serum from pregnant women and multitransfused individuals, setting the stage for the elucidation of the major histocompatibility complex (MHC) in humans (4). Over the past ten years, researchers converging from these fundamental observations have provided considerable insight into the immunogenetics of autoimmune endocrine disorders. Here we review primarily the relation between genetic variation in the MHC and autoimmune thyroid disease.

The Major Histocompatibility Complex

Structure, Polymorphism, and Tissue Distribution. The MHC, located on the short arm of chromosome 6, is undoubtedly the best-mapped region (5, 6) of the human genome (Fig. 1). In the nomenclature adopted by the eighth International Histocompatibility Workshop (7), class II antigen loci are designated DP (previously SB), DQ (previously DC), and DR (Tables 1, 2).

In theory more than a half-billion different combinations (haplotypes) of the known alleles (Table 3) are possible, but considerably fewer are actually observed. Genes of the three antigen classes are sufficiently close together that haplotypes are generally inherited as units within families; recombination within MHC haplotypes is uncommon. Specific combinations of alleles are much more frequent than expected (linkage disequilibrium).

Class I antigen molecules are made up of a polymorphic heavy chain (~45,000 daltons) and a monomorphic β_2 microglobulin (~12,000 daltons), which seems to be essential for insertion of class I antigens into the plasma membrane and contributes significantly to the antigenicity of the second domain of the heavy chain (5, 6).

Class II antigen molecules, on the other hand, comprise a heavy α chain and a β chain; the β chain is the more polymorphic in the case of DR, and both chains contribute to the polymorphism of DQ (8). Certain HLA-DR and DQ alleles are in linkage disequilibrium; DP alleles are not.

Class I antigens are found on all nucleated cells. Class II antigens are less widely distributed; they have been described on B cells, macrophages, and dendritic cells, and seemingly can be induced on lymphoid and nonlymphoid cells (9–12). Under certain circumstances class II antigens may be expressed in other tissues in which they are not normally found; this is true for the thyroid (13), an observation of immediate interest here. It is not presently known whether only DR can be so expressed or whether all class II antigens are coordinately expressed, whether this is primary or secondary to autoaggressive immunologic damage, and whether the density of these aberrantly expressed molecules varies among in-

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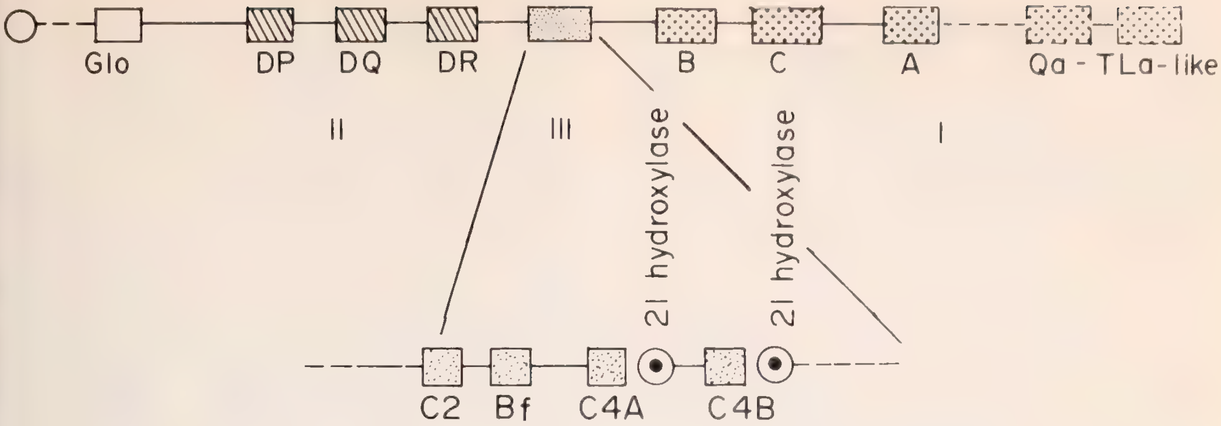

Fig. 1. Location of principal genes mapped to the human major histocompatibility complex, 1984.

dividuals. McDevitt's group recently suggested that class IV antigen expression occurs, seemingly coordinately with that of class II antigens (14); little is known about the distribution or function of class IV antigens.

Function. The MHC seems to be involved in the recognition of nonself, directing and restricting the immune response (6). This and other functions seemingly involve cell-to-cell contact and the control of traffic into specific tissues. There is some evidence that, in the mouse, a set of class I antigens may act as embryonic tissue markers, or differentiation antigens. The MHC also influences levels of sex steroids by influencing the levels of their transport proteins, the density of their receptors, and target tissue responses. Given a choice, mice tend to seek out mates heterozygous at H2 (mouse MHC equivalent) loci; pheromones may control this.

MHC antigens are also involved in transducing the message mediated by cyclic AMP-dependent hormones, and may act as regulators of receptor affinity; class I polymorphism apparently influences variation in these two aspects of

TABLE 1
Recognized HLA Specificities

HLA-A	HLA-B		HLA-C	HLA-D	HLA-DR	HLA-DQ	HLA-DP
A1	B5	B49(21)	Cw1	Dw1	DR1	DQw1	DPw1
A2	B7	Bw50(21)	Cw2	Dw2	DR2	DQw2	DPw2
A3	B8	B51(5)	Cw3	Dw3	DR3	DQw3	DPw3
A9	B12	Bw52(5)	Cw4	Dw4	DR4		DPw4
A10	B13	Bw53	Cw5	Dw5	DR5		DPw5
A11	B14	Bw54(w22)	Cw6	Dw6	DRw6		DPw6
Aw19	B15	Bw55(w22)	Cw7	Dw7	DR7		
A23(9)	B16	Bw56(w22)	Cw8	Dw8	DRw8		
A24(9)	B17	Bw57(17)		Dw9	DRw9		
A25(10)	B18	Bw58(17)		Dw10	DRw10		
A26(10)	B21	Bw59		Dw11(w7)	DRw11(5)		
A28	Bw22	Bw60(40)		Dw12	DRw12(5)		
A29(w19)	B27	Bw61(40)		Dw13	DRw13(w6)		
A30(w19)	B35	Bw62(15)		Dw14	DRw14(w6)		
A31(W19)	B37	Bw63(15)		Dw15			
A32(w19)	B38(16)	Bw64(14)		Dw16	DRw52		
Aw33(w19)	B39(16)	Bw65(14)		Dw17(w7)	DRw53		
Aw34(10)	B40	Bw67		Dw18(w6)			
Aw36	Bw41	Bw70		Dw19(w6)			
Aw43	B42	Bw71(w70)					
Aw66(10)	B44(12)	Bw72(w70)					
Aw68(28)	B45(12)	Bw73					
Aw69(28)	Bw46	Bw4					
	Bw47	Bw6					
	Bw48						

Antigens followed by a number in parentheses are recognized as splits of the antigen in the parentheses; for instance, A23 is a split of A9.

TABLE 2
New Provisional HLA Specificities

New	Previous equivalents
Aw66	LN
Aw68	A28, negative to A2/28
Aw69	A2/28
Bw64	B14.1, (8w63)
Bw65	B14.2, (8w62)
Bw67	SN-2, Te90, (8w57)
Bw70	Bu + SV, K5, Da(6), (8w59)
Bw71	Bu
Bw72	SV
Bw73	KA, IEH
DRw11	LB5
DRw12	LB5 × 8, DR5 short, FT23
DRw13	6.6, 6.1 6Z
DRw14	6.9, 6.3, 6X, 901
DRw52	MT2
DRw53	MT3
DQw1	DC1, MB1, MT1, LB-E12
DQw2	MB2, DC3, Te24, LB-E17
DQw3	MB3, MT4, DC4, TB21
DPw1	SB1, PL3A
DPw2	SB2
DPw3	SB3
DPw4	SB4, PL3B
DPw5	SB5
DPw6	SB6
Dw13	DB3
Dw14	LD40
Dw15	DYT, YT
Dw16	DB8, B8
Dw17	7A, (Dw7A)
Dw18	6A, (Dw6A)
Dw19	6B, (Dw6B)

hormone action. In some instances class I molecules actually act as receptors. We consider unlikely the recent suggestion that the class I heavy chain antigen is a subunit of the insulin receptor (15); H2 antigen may, however, become associated with the insulin receptor (16) in membrane patches, and may well influence the modulation of insulin action (15). It is not known why the loci for 21β-hydroxylase enzymes (17, 18) and testicular phosphoglycerate kinase (19) are in the MHC; this may be related to reproductive fitness.

Linkage and Association. The distinction between the genetic concepts of linkage and association should be made clear (5, 6). Linkage indicates the cosegregation of alleles at two loci located close together on a chromosome. For instance, 21β-hydroxylase deficiency is a recessive disorder closely linked to HLA. This deficiency always cosegregates with the same two HLA haplotypes in a particular family, but may cosegregate with different haplotypes in other families (Fig. 2). Linkage implies no permanent coupling of the disease allele with any particular HLA allele. Recombination occurs, at a rate dependent on the distances between these alleles.

By contrast, association implies the co-occurrence of particular HLA phenotypes and particular diseases, demonstrated statistically. Association of a disease with a specific HLA allele in a variety of populations may be due to tight linkage of a disease-susceptibility allele to HLA (linkage disequilibrium). For instance, the gene for 21β-hydroxylase deficiency is in linkage disequilibrium with HLA-Bw47 in caucasoids. A genuine influence of the HLA allele on disease susceptibility may underlie the association. For instance, association might arise through nonspecific facilitation of the immune response by an immune-response gene linked to an HLA allele, or through genes controlling the activity of genes at other loci (epistasis).

Just as haplotypes may distinguish racial groups better than do single HLA alleles (20), evidence is emerging that complete haplotypes may be more strongly associated with certain diseases than are single alleles (21, 22).

In many autoimmune disorders in which associations with HLA have been noted, including thyroid disorders (but excluding subacute thyroiditis), only 40–60% of patients possess the associated HLA allele (5, 6, 20). Several factors may account for this:

- Association reflecting linkage disequilibrium of "marker" alleles with nearby alleles influencing susceptibility
- A major role of the environment in the development of the disease
- Involvement of genetic factors other than HLA
- Disease heterogeneity, HLA association being relevant in only a subset of cases

TABLE 3
Number of Recognized Alleles of the Major Histocompatibility Complex (1984)

	Class I			Class II				Class III			
Locus	A	B	C	D	DR	DQ	DP	Bf	C2*	C4A*	C4B*
No. of alleles	20	47	8	16	19	3	6	15(4)†	5(2)	11(5)	14(4)

* Including null alleles.
† Figures in parentheses are numbers of rare alleles, included in total.

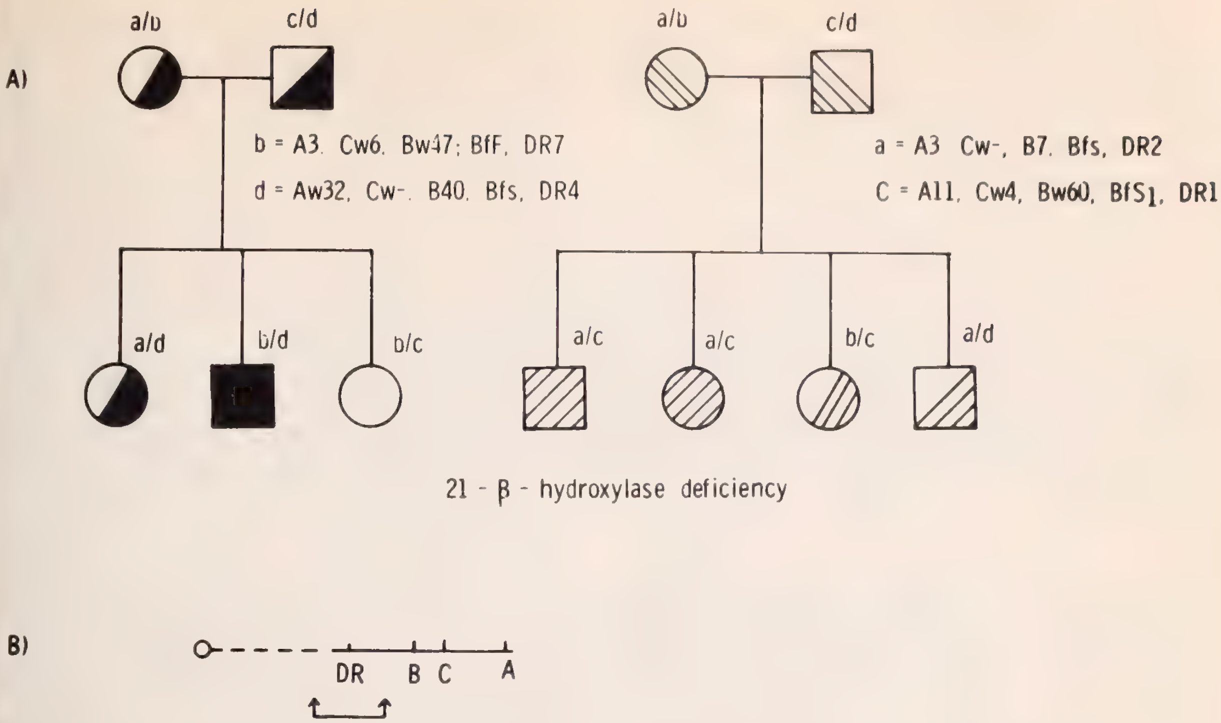
A)
a/b
c/d
b = A3, Cw6, Bw47; BfF, DR7
d = Aw32, Cw-, B40, Bfs, DR4
a/d
b/d
b/c
a/b
c/d
a = A3 Cw-, B7, Bfs, DR2
C = A11, Cw4, Bw60, BfS1, DR1
a/c
a/c
b/c
a/d
21 - β - hydroxylase deficiency
B)
DR
B
C
A

Fig. 2. Linkage versus association. A Two hypothetical families with 21β-hydroxylase deficiency, an HLA-linked recessive disorder. (○) Females; (□) males. Half-filled symbols signify carriers, filled symbols signify affected family members. Letters a to d denote HLA haplotypes; only disease-linked haplotypes are shown. B Association of autoimmune disease with DR alleles may be related to DR-linked disease-susceptibility genes, or to a class II antigen gene distinct from DR which cannot be typed for but which maintains a degree of linkage disequilibrium with DR alleles. Other possibilities are discussed in text.

Nonspecific Role of HLA-DR3. Several autoimmune organ-specific diseases, including a number of endocrine disorders, are associated with HLA-DR3. This suggests that HLA-DR3 is related to a general, broad defect in immunoregulation. Disease-free HLA-DR3-positive individuals have been reported to have reduced suppressor T cell function, inefficient induction of presuppressor T cells, and B cell hyperactivity (23–26) (Table 4). Moreover, their macrophages process antigens much more slowly than those of DR3-negative persons, a characteristic associated in experimental animals with enhanced immune responsiveness (27, 28). Some of these reports have been challenged. Clearly, studies of in vitro immune function in HLA-DR3-associated disorders can be interpreted only if healthy DR3-positive controls are also studied.

Graves' Disease and HLA

A review of studies of Graves' disease shows how HLA associations illuminate the natural history and genetics of autoimmune thyroid disease.

Graves' disease is primarily associated with HLA-DR alleles: HLA-DR3 in caucasoids and Chinese and HLA-Dw12 in Japanese (5, 6).

In Japanese, probands in families with several affected members have exhibited HLA-DR5 more often than expected (29). More recently, in expanded family studies, no difference in frequency of HLA antigens was observed between probands and healthy control populations (30). The significance of these findings remains to be determined.

TABLE 4
Characteristics of Healthy HLA-DR3-Positive Individuals

Increased skin reactivity and lymphocyte reactivity to antigens
Decreased rate of processing of sheep red blood cells by macrophages
Decreased Fc receptor-mediated clearance of IgG-sensitized red blood cells
Decreased Tγ cell numbers
Decreased concanavalin A (Con A) induced suppressor cell activity
Decreased response of T cells to suboptimal concentrations of phytohemagglutinins and Con A
Increased spontaneous immunoglobulin-secreting B cells
Increased synthesis of IgG by B cells stimulated by pokeweed mitogen in the presence of glucocorticoids

A suggestion that a variant of hyperthyroidism with diffuse goiter and without ophthalmopathy is associated with HLA-DR5 in iodide-deficient areas (31) has not been confirmed (32).

Studies based on the established association of Graves' disease with HLA-B8 and DR3 have been more fruitful.

B8 and DR3. We have suggested (33, 34) that the HLA-linked susceptibility to Graves' disease is dose-dependent, that is, that HLA-B8 homozygotes are at greater risk than heterozygotes. Later studies in Chinese and Hungarian families indicated that specific HLA-B heterozygote combinations, always involving the disease-related allele (such as B8/B35 in Hungarians and Bw46/B13 and Bw46/B40 in Chinese), conferred increased risk for Graves' disease (6). In contrast to the dose effect suggested by HLA-B genotypes, neither HLA-DR3 homozygotes nor heterozygotes comprising DR3 and another specifiable HLA-DR antigen were more frequent than expected in patients with Graves' disease (34). These observations may be explained in two ways.

First, an HLA-DR allele which cannot as yet be typed for conventionally might interact with HLA-DR3 to increase disease susceptibility. (The proportion of DR "blanks," or instances of untypability, allows for several as-yet-untyped DR antigens.)

Second, disease susceptibility might be related to another class II antigen. A likely candidate is the DQ locus, which is equivalent to the mouse IA region. This suggestion will be developed in the next section, on the genetics of Graves' disease.

Positivity for HLA-DR3 in patients with Graves' disease has been correlated with a number of biochemical and clinical factors. These include younger age of onset (34), ophthalmopathy (34, 35), poor response to antithyroid drugs (34–38) and resistance to ^{131}I treatment (34), the presence of lymphocytotoxic antibodies (39), T lymphocyte proliferation in response to thyroglobulin (40), hypergammaglobulinemia G (41), reduced serum C4 levels (42), and continued depression of OKT8+ lymphocytes after antithyroid drug therapy (compared to DR3 negatives, whose OKT8+ lymphocyte subsets return to normal) (43).

Clinical Course. Indications that hyperthyroidism in patients with HLA-B8 (34, 36) and HLA-DR3 (34, 38) was less likely to remit following adequate courses of antithyroid drugs could not be confirmed in retrospective studies (44–45) or in a prospective study (46) in which all the patients were treated and assessed at uniform intervals. Among other reasons, differences in the thionamide used, dosages, and duration of therapy could account for these divergent results. Both HLA phenotypes and anti-thyroid-stimulating hormone (TSH) receptor antibody status were examined in a group of patients with Graves' disease, as possibly predictive of the need for more than one dose of ^{131}I; persistence of anti-TSH receptor antibody after treatment was found to be the major predictor of a need for further doses of ^{131}I; HLA-DR3 was of marginal predictive value (47). It has been reported that patients with Graves' disease who are treated with radioactive iodine are more likely to remain euthyroid for longer periods of time if they are DR3 positive, even with higher titers of antimicrosomal antibody (48; unpublished data). This suggests inherent resistance of the thyroid cells of HLA-DR3-positive individuals to radiation damage.

Cluster Analysis. Initial attempts to correlate individual characteristics of Graves' disease patients with their HLA phenotypes have not been unequivocal; see discussion above and (49). For this reason, characteristics of 196 patients with Graves' disease were evaluated by cluster analysis (35). A mathematical measure of similarity was obtained with respect to 49 attributes, including HLA-A and HLA-B antigens, clinical course of disease after withdrawal of antithyroid drugs, ophthalmopathy, thyroid autoantibodies, blast transformation in response to thyroglobulin, circulating immune complexes, short-lived and concanavalin-induced suppressor T cell activity, and lymphocytic infiltration of the thyroid gland.

Three subgroups were distinguishable (Fig. 3). Group I included patients with small goiters, little ophthalmopathy, and a tendency to remission following treatment with antithyroid medications; group IIa showed clinical and laboratory features of hashitoxicosis; and group IIb comprised patients with severe disease, associated ophthalmopathy, positive family history, and marked evidence of autoaggression. It is of note here that 11% of group I, 21% of group IIa, and 87% of group IIb were positive for HLA-B8. (A group of 117 patients typed for HLA-DR show a similar trend for HLA-DR3 [Frecker, 50]). Thus, cluster analysis identified clinically relevant groupings of patients, the severity of whose disease (as evidenced by tendency to relapse) correlated with possession of HLA-B8.

The same data were recently reanalyzed using a different cluster analysis method,

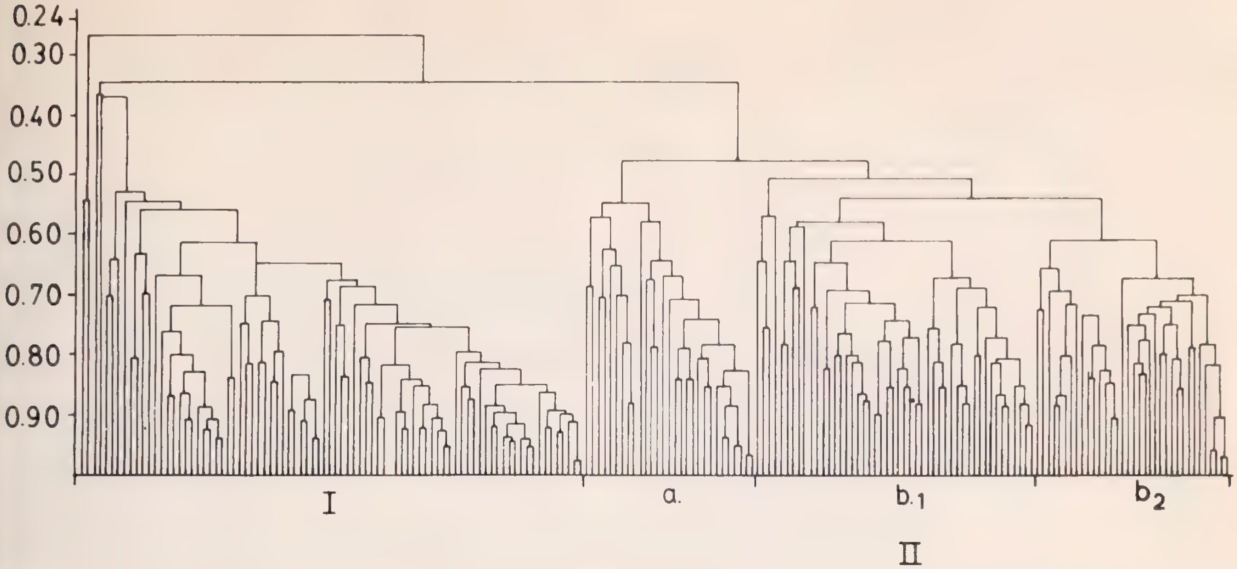

Fig. 3. Result of cluster analysis in form of binary tree. Scale of similarity on *y* axis indicates resemblance of cases to one another. Cases are linked by criterion of mutually highest resemblance to one another, and clusters are progressively joined by criterion of highest average correlation between members of separate clusters being joined. Most initial pairs of patients are similar at 0.8 level or above; groups I and II join at 0.35 level; groups IIa and b branch at 0.48 level. Individual cases are arranged along *x* axis in groups I to IIb2. A residuum of four cases is shown at extreme left, dissimilar to one another and to all other cases. Reproduced, with permission, from Stenszky et al (35).

CLUSTAN (50). Two groups were again indicated, corresponding to groups I and II of the earlier study. Only 7 of 196 patients were assigned differently in the two analyses. Both analyses agree in indicating the same attributes, with the exception of familial aggregation, as discriminative. In the CLUSTAN group II, 81% of patients were HLA-B8, compared to 70% in the first analysis. These largely concordant results demonstrate the power of cluster analysis in identifying patient subsets, as well as the value of HLA typing in conjunction with clinical and laboratory features in identifying patients likely to respond well to adequate courses of antithyroid medication. In a more limited analysis, Dahlberg and his colleagues related HLA-DR3 positivity, in association with age of onset and sex, to low likelihood of remission after a course of antithyroid medication (51). This provides independent support for the contention that patient groups can be discriminated more powerfully by considering several factors together rather than single factors alone.

Genetics: Recessive Inheritance? That Graves' disease is familial has been appreciated almost as long as the disorder has been recognized (49). A large Newfoundland pedigree (Fig. 4) illustrates the familial aggregation of both Graves' disease and autoimmune thyroiditis (52).

The increased risk of Graves' disease among HLA-B8 homozygotes, and among heterozygotes for the HLA-B allele associated with Graves' disease in an affected relative and another specific B allele, suggested early on that more than one HLA-linked gene influenced susceptibility to Graves' disease (6, 33, 34). This suggestion was confirmed when HLA haplotype sharing was examined in 67 affected sib pairs (53); 41% of the sib pairs shared two HLA haplotypes, 23% one haplotype, and 3% no haplotype, a distribution quite different from that expected (Table 5). The observed sib-pair haplotype distribution was compared with those expected for recessive and dominant inheritance of susceptibility, over a wide range of frequencies of susceptibility gene. Dominant inheritance could be discounted because observed and expected numbers of sib pairs sharing two, one, and no haplotypes were consistently quite different; the lowest chi-square value for goodness of fit between observation and expectation was 6.7 at a gene frequency of 0.05. On the other hand, for recessive inheritance, a range of gene frequencies from 0.2 to 0.4 gave expectations not significantly different from the observed distribution ($p > 0.05$).

Further analyses required knowledge of the recurrence rate of Graves' disease in first-degree relatives of unselected probands. We found the overall sib recurrence rate to be 2.9%; the highest recurrence rates were among mothers (5.1%) and sisters (6.5%) of female probands. Sisters of affected males (1.8%) were more often affected than their brothers (0.0%). With a sib recurrence rate

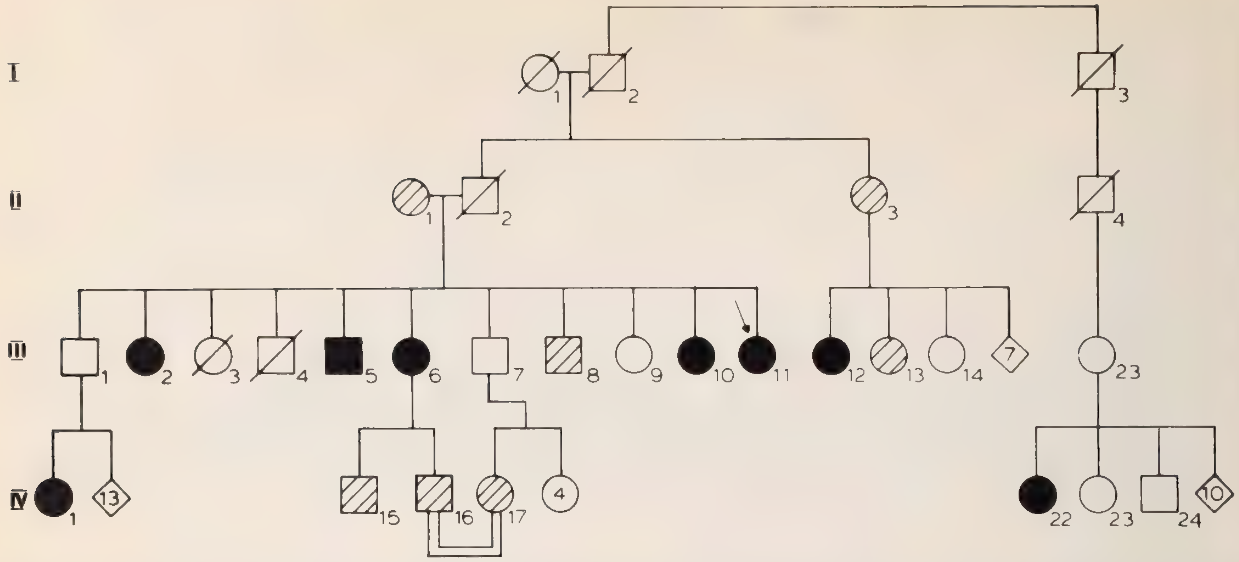

Fig. 4. Large Newfoundland family having many members with autoimmune thyroid disease. (●, ■) Graves' disease; (○, □) Hashimoto's thyroiditis; (n, n) persons not examined, or examined and found normal; (16–17) cousin marriage. Reproduced, with permission, from Farid et al (52).

of 2.9% and 63% of affected sibs sharing both HLA haplotypes, it can be estimated that 7.2% of sibs HLA-identical to a proband will develop Graves' disease. By comparison, 4% of sibs sharing one haplotype and 0.5% of sibs sharing no haplotype are expected to develop Graves' disease.

The value of 7.2% also represents the lower limit of genotype penetrance (54). Assuming a recessive gene with a frequency of 0.3 and the population disease frequency of 0.65% also implies a genotype penetrance of 7.1%. The sib pair haplotype data, however, could accommodate a penetrance as high as 16%. Such analyses must be interpreted tentatively; relatively few sib pairs were genotyped, and these calculations do not differentiate between true recessive and complex interactions of nonallelic genes mapping inside the MHC. With recessive inheritance, the distribution of HLA genotypes in unselected patients should be in Hardy-Weinberg proportions. The observed proportions of HLA-B8 homozygotes and heterozygotes correspond very closely to this expectation (Table 6). This finding again favors a recessive HLA-linked genetic contribution to Graves' disease susceptibility over other gene dosage models.

TABLE 5

HLA Haplotype Distribution among Sib Pairs with Graves' Disease

	Shared haplotypes		
	2	1	0
Observed	41	23	3
Expected*	16.75	35.5	16.75

* Comparison between observed and expected distributions yielded χ^2_1 value of 50.8.

Morever, there is no evidence of an increase in HLA-DR3 homozygosity or of heterozygote phenotypes comprising DR3. In addition, some 75% of B8-positive individuals are DR3-positive. This analysis of B8 thus should approximate DR3 findings. Again, the possibility of complementation between two genes in the MHC is not excluded. This complementation could take the form of the generation of hybrid (α and β chain) class II antigen molecules. Susceptibility to Graves' disease is clearly different from susceptibility to type I diabetes, in which HLA-DR3/DR4 heterozygotes are 15–20 times more susceptible than either homozygote (54). An intermediate or gene-dose model (55) in which heterozygosity for disease allele is also associated with increased risk seems improbable.

On the basis of population prevalence, preponderance of disease among females, and HLA

TABLE 6

HLA-B8 Genotype Distribution in 256 Patients with Graves' Disease

	B8/B8	B8/BX	BX/BX
Observed	20	95	141
Expected*	17.4	98.02	140.94

* On the basis of Hardy-Weinberg equilibrium and a B8 allele frequency of 0.258; $\chi^2 = 0.607$.

data, we have calculated the probabilities of manifestation of Graves' disease (Table 7) for specific genotype and sex combinations; these probabilities remain to be empirically verified.

Other Genetic Influences. The discrepancy between the concordance rate of monozygotic twins—reportedly 50% (49), albeit probably inflated by selection bias— and these estimates of MHC-related genotype penetrance suggests additional genetic influences on susceptibility to Graves' disease (49).

We reported an association between immunoglobulin heavy-chain markers and Graves' disease (56, 57), and recently confirmed this observation with data from an eastern Hungarian population (58). The available data show that homozygosity for the common Gm haplotype (fb) is associated with greater susceptibility than heterozygosity, suggesting that immunoglobulin-associated susceptibility, like MHC-associated susceptibility, is dose dependent. Our earlier observations have been extended in Japanese family studies; the most recent of these showed that 37 of 38 affected sib pairs shared an HLA and a Gm haplotype, confirming the importance of immunoglobulin heavy chains as well as HLA in susceptibility to Graves' disease (29, 30). It is difficult to accommodate the findings for sibs sharing only one HLA and one Gm haplotype in these Japanese family studies, and the general finding that some two-thirds of affected sib pairs (including Japanese sib pairs) are HLA identical (59, 60). More sophisticated analyses may resolve this problem. Interactions between Gm and HLA have been demonstrated in several experimental systems and disease liability studies (57), which prompts us to suggest that Gm haplotype sharing may well be relevant only in patients who share at least one HLA haplotype.

We have suggested that the interactions of genetic factors in Graves' disease susceptibility are probably complex (49). Some factors appear to contribute independently, some to interact multiplicatively, and some to cancel the influence of others. An environmental trigger may well be required to precipitate disease, as indicated by the observation that not all sibs who are HLA identical and share one HLA and one Gm haplotype with the proband develop Graves' disease (30).

TABLE 7

Probability of Graves' Disease for Combinations of Sex and HLA Marker Antigen

	Probability (%) with HLA marker antigen			
Sex	B8+	B8−	DR3+	DR3−
Female	2.47	0.71	3.00	0.62
Male	0.62	0.18	0.75	0.16
If other relative affected				
Female	11.44	3.26	14.91	2.86
Male	2.86	0.82	3.73	0.72

Family studies can also be used to infer MHC haplotypes associated with Graves' disease, because these studies allow the components of "susceptibility" haplotypes to be identified directly, without the need to invoke linkage disequilibrium (53). Frequencies of haplotypes thus found in probands with Graves' disease can be compared to frequency in healthy families (53). This approach again implicates the HLA-DR region of the haplotype as the one most relevant to disease susceptibility (Table 8).

On the other hand, certain complotypes (combinations of alleles at loci in the MHC which code for components of the complement pathways and class I and II antigens; see Table 3), including some rare complotypes, are found in persons with

TABLE 8

HLA-A1, Cw3, B8, DR3 Haplotypes and Haplotype Segments in 33 Graves' Families Compared to 56 Healthy Families

Haplotype*				Families					
				Graves'		Controls			
A	Cw	B	DR	No.	%	No.	%	RR†	EF‡
1	3	8	3	9	10.2	12	5.4	2.01	0.051
	3	8	3	11	12.5	13	5.8	2.31	0.071
		8	3	20	22.7	14	6.3	4.41	0.176
			3	31	35.2	19	8.5	5.87	0.292

* Total number of haplotypes: in patient families, 88; in control families, 224.

† RR, relative risk.

‡ EF, etiological fraction = (RR − 1)/RR hp, where hp is haplotype frequency in patients. EF indicates strength of association of liability with HLA haplotypes or haplotype segments.

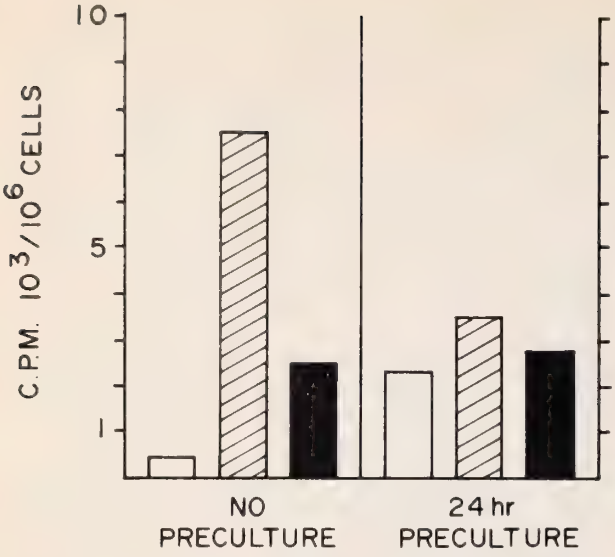

Fig. 5. Influence on specific suppressor T-cell function of preculture of peripheral mononuclear cells for 24 hr. (□) Culture of lymphocytes of healthy twin; (▨) culture of lymphocytes of twin with Graves' disease; (■) coculture of lymphocytes of both twins. Results are expressed as counts/min of $[^3H]$thymidine taken up by mononuclear cells in response to culture for seven days with thyroid membrane antigens (TMA). Preculture for 24 hr removes adherent cells (mainly monocytes; some T lymphocytes). Results imply (**left**) that T lymphocytes of twin with Graves' disease, but not of healthy twin, are sensitized to TMA. When adherent cells were removed (**right**), sensitization to TMA was demonstrated in unaffected twin's lymphocytes. Surprisingly, $[^3H]$thymidine uptake by lymphocytes of affected twin was reduced after removal of adherent cells. This fraction contains accessory cells necessary for T-cell-mediated immune responses, as well as suppressor cells. Depending on balance between these two populations, removal of adherent cell fraction results in enhancement (healthy twin) or reduction (Graves' twin) in T-cell response. Reproduced, with permission, from Balazs et al (62).

Graves' disease more commonly than in unaffected persons (22, 49, 52). This does not necessarily absolve DR; susceptibility seemingly maps to the class II region, and certain unusual haplotype combinations of DR and complement alleles, which are relatively stable, tend to be associated with Graves' disease (21, 61).

HLA-DR3 and Thyroid-Stimulating Antibody. In our investigations of a pair of identical twins, one with Graves' disease and the other with no evidence of thyroid disease (62), we found that the twins shared the HLA haplotype A1, B8, Cw−, DR3. The proband showed good T-cell-proliferation response to thyroid membrane antigens, as measured by $[^3H]$thymidine uptake. When adherent cells were removed from the peripheral leukocytes of the unaffected twin, her T cells proliferated well above background in response to thyroid membrane antigens, suggesting that she also has autoreactive lymphocytes, which have been kept in check by suppressor cells (Fig. 5). We wondered whether this finding might be generalizable to all HLA-DR3-positive individuals. It was therefore intriguing to learn of experiments comparing the ability of immunoglobulin G from HLA-DR-typed healthy individuals to stimulate the generation of thyroidal cyclic AMP (63). Immunoglobulin G from HLA-DR3-positive persons caused greater stimulation of cyclic AMP than those from HLA-DR-negative persons. This implies that HLA-DR3-positive persons have higher levels of thyroid-stimulating antibody.

Genetics in Graves' Disease. In sum, evidence is emerging of a broad autoimmune effect conferred by DR3 which, added to the effect of specific immune-response genes linked to HLA and immunoglobulin, contributes to susceptibility to Graves' disease. Female sex certainly, and other genes of minor effect in an appropriate environment probably, also increase the probability of Graves' disease. Clearly, persons with most but not all of these factors can also develop Graves' disease.

Other Thyroid Diseases

HLA typing has demonstrated that autoimmune goitrous thyroiditis is associated with HLA-DR5, and that primary myxedema is associated with HLA-DR3 (57, 64). Bliddal et al (65) have reported a relation in goitrous persons between TSH-receptor modulatory antibodies and DR5. Using more specific HLA-DR typing sera, we have recently found that 60% of patients with Hashimoto's thyroiditis were HLA-DR4-positive; the prevalence of HLA-DR5 was decreased in the patient population (C. Thompson and N. R. Farid 1985, unpublished observations).

HLA typing has also showed that subacute (de Quervain's) thyroiditis is associated with HLA-B35 and not with HLA-DR antigens in some 90% of patients, suggesting that these patients' T cells recognize the combination of a thyrotropic virus and B35, which mediates thyroid cell killing (64, 66).

By contrast, transient thyroiditis, both postpartum and other, has been variously associated with HLA-DR3, HLA-DR5, and HLA-DR4. The DR4 association was reported from Europe, where it is unusual for hypothyroidism to be preceded by a thyrotoxic phase (67, 68). Of more than 30 patients typed in St. John's, Newfoundland, more than half had HLA-DR4. Also, we have the impression that HLA-DR3/DR4 heterozygotes are overrepresented among patients with non-postpartum thyroiditis. Reported differences in HLA phenotypes may therefore reflect differ-

TABLE 9

HLA-DR7 in 66 Patients with Epithelial Carcinoma of Thyroid

Type of Ca	No.	% HLA-DR7-positive
All: radiation-related and nonradiation-related[*]	66	36.3%
Nonradiation-related	42	45.2%
Follicular	7	86.7%
Mixed papillary and follicular	14	28.7%
Papillary	7	28.6%

[*] HLA-DR7 was associated with earlier onset of disease in radiation-related carcinoma.

Adapted from Sridama et al (74).

ences in clinical entities studied and in ethnic origins of patients. More likely, some of the sera used for typing were cross-reactive with HLA-DR4. The specifications of HLA-DR4 and HLA-DR5 remain problematic, despite the better definitions achieved in the Ninth Histocompatibility Workshop.

We found that silent thyroiditis unrelated to pregnancy occurred in an unexpectedly large number of women taking lithium carbonate for bipolar depression; these women also showed an increase in HLA-DR4.

Both HLA-DR4 and HLA-DR3 are commonly encountered in women with Hashimoto's thyroiditis combined with premature ovarian failure.

A relation seems also to hold between HLA alleles and congenital hypothyroidism (69, 70). Japanese workers have found an increased frequency of Aw32 in mothers of babies with hypothyroidism related to ectopic thyroid (70). Mothers of babies having thyroid dysgenesis associated with thyroid growth-blocking antibodies were found to be HLA-DR3-positive disproportionately often (71). The interference of these antibodies with organogenesis suggests a mechanism similar to that in findings of congenital heart block in children of women with collagen vascular disease (72).

Finally, several studies show an association of HLA-DR antigens and differentiated thyroid carcinoma. In southern Italy an increase in HLA-DR1 was shown in patients with thyroid carcinoma (73). Sridama et al (74) have demonstrated an increase in HLA-DR7 in patients with epithelial cell carcinoma (Table 9), and we note an increase in HLA-DR7 in a limited number of patients with medullary carcinoma of the thyroid (Table 10, Fig. 6). It is intriguing that HLA-Dw7 has been found to be common in patients with testicular embryonic tumors (75). Perhaps the specific class II antigens with which these malignancies are associated confer defective immunological surveillance, which allows malignant cells to proliferate into clinically detectable tumors. It is unlikely that MHC-coded differentiation antigens underlie these associations because class I rather than class II antigens are more often associated with differentiation.

Summary

HLA typing has contributed to thyroid medicine in several significant ways:

- By refining the differentiation of disease entities.
- By providing markers indicating likely prognosis and severity of the disease.
- By elucidating the genetic basis of disease susceptibility.
- By elucidating underlying pathological processes.

TABLE 10

HLA Antigens in Unrelated Patients with Medullary Thyroid Cancer (MTC)

Patients[*]	HLA				CT level[†] (pg/ml)	Disease stage[‡]
	A	B	C	DR		
1	3,9	12, –	4, –	2,7	1000	As
2	2,3	7,13	1,3	4,7	100	As
3 +	1,10	22,35	4, –	– ,7	675	Bs
4	2,3	15, –	2, –	4,7	30	As
5 + +	1,11	35,13	4, –	1,7	100	As
6	1,2	7,40	– ,	4,7	70	As
7 + + +	1,10	8,12	3, –	6,7	480	Bs
8	1,11	8,18	5, –	1,3	100	As
9 + + + +	3, –	7,14	– , –	2,7	1300	Bs

[*] +, mother affected with MTC; + +, sister affected with MTC; + + +, father affected with MTC; + + + +, mother and aunt affected with MTC.

[†] CT, calcitonin.

[‡] As, after surgery; Bs, before surgery.

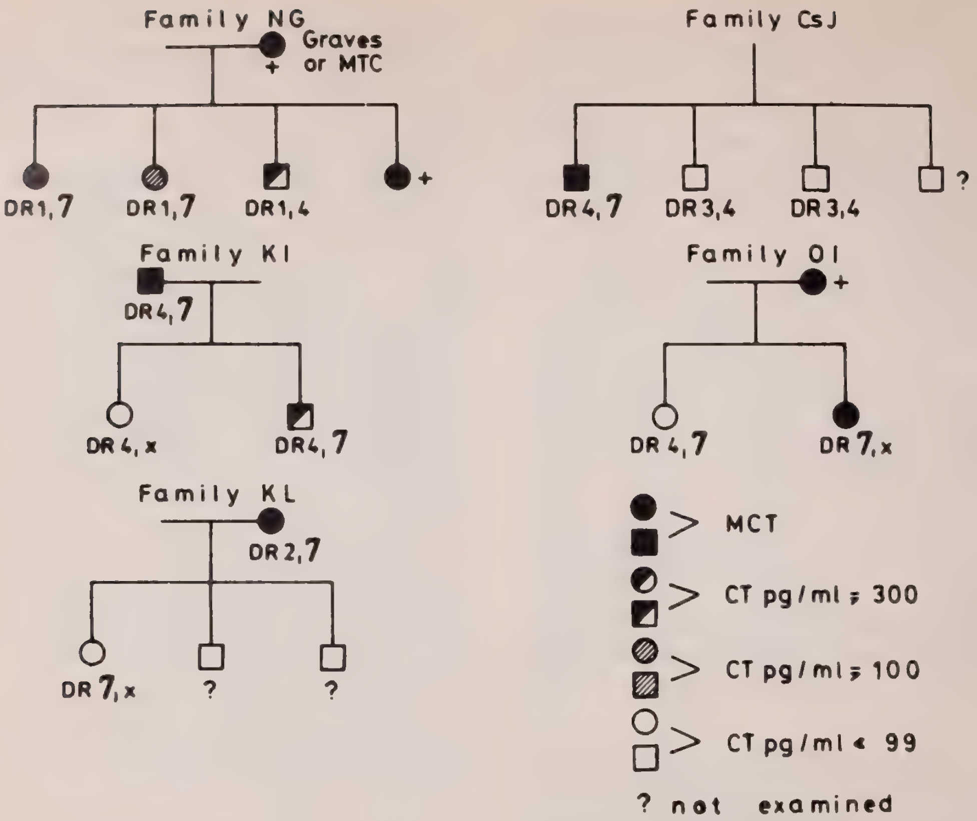

Fig. 6. Family study, medullary thyroid cancer. (X) deceased; (?) status unknown; CT, calcitonin; MCT, medullary carcinoma thyroid.

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Genetics of Endocrine Autoimmunity: Animal Models

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Animal models of autoimmune disease have provided tremendously valuable leads in the study of the genetic basis of autoimmune diseases in humans; in our view this is the greatest value of such models. Those of us who study a particular animal model have always to be aware that what we are observing may be only a limited reflection of the entire universe of human autoimmune diseases, and if we study a different model we may get a rather different view of things. This paper presents a selective review of the genetic basis of autoimmune disease and of the contribution animal models have made to our understanding. In the final analysis, the best model of human autoimmune disease is the patient who actually has one of these diseases. For that reason this paper extends to human data.

Autoimmunity: Development of Concepts

The mid 1950s was indeed an important period for the field of autoimmunity. In London, Doniach and Roitt found autoantibodies against thyroglobulin in patients with Hashimoto's disease (1). In Buffalo, Witebsky and Rose induced autoimmune thyroiditis in rabbits (2). These two discoveries broke the 50-year-old stranglehold of the false dogma that autoimmunity was impossible. In Dunedin, New Zealand, Adams and Purves discovered in the serum of patients with Graves' disease a substance which they called long-acting thyroid stimulator, subsequently proved to be an autoantibody which causes abnormal stimulation of the thyroid gland (3–5). In Pasadena, Jerne made what is probably the single greatest contribution to understanding of the immune system since its discovery. He realized that antibodies are not built to fit an invading antigen, but are preformed in enormous variety like ready-made suits awaiting a customer (antigen) to fit (6).

Forbidden Clone Theory. Macfarlane Burnet extended Jerne's radical new concept by realizing that antibodies must come from cells and that a single clone of cells must make antibody of a single specificity (7). The clone is therefore the unit of specificity in the immune system. From his many years of experience studying mutation in bacteria, Burnet realized the importance of mutations in rapidly dividing cells in generating diversity.

In addition, Burnet postulated that somatic mutation could give rise to autoimmunity by creating new clones with specificity for self-antigens, and he coined the term "forbidden clones" for clones guilty of such a mistaken identification of their target (7).

Burnet's theory provides a ready explanation for many of the observed features of autoimmune diseases, including the random age of onset, the increase in variety of symptoms with age, and the observation that only approximately 50% of monozygotic twins are concordant for autoimmune diseases (8). Furthermore, in those instances of concordance in monozygotic twins, the age of onset of disease in the two twins can be widely disparate. We also observe this phenomenon in New Zealand Black mice. The age of onset of autoimmune hemolytic anemia is widely variable in these mice, even though they are genetically identical and even when they are housed together in large groups so that environmental factors such as intercurrent infections are standardized. We attribute this phenomenon to the

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need for appropriate somatic mutations to generate clones with the particular specificity needed for self-reaction.

Genetic Predisposition

Burnet's theory in itself offers no explanation for the familial tendency of autoimmune diseases. In theory, the tendency for particular autoimmune diseases to run within families could be due to sharing of environmental conditions rather than to genetic mechanisms. However, twin studies have revealed a much higher concordance rate in monozygotic twins than in dizygotic twins for several autoimmune diseases, including Graves' disease and insulin-dependent diabetes, making it clear that genetic factors are responsible (8).

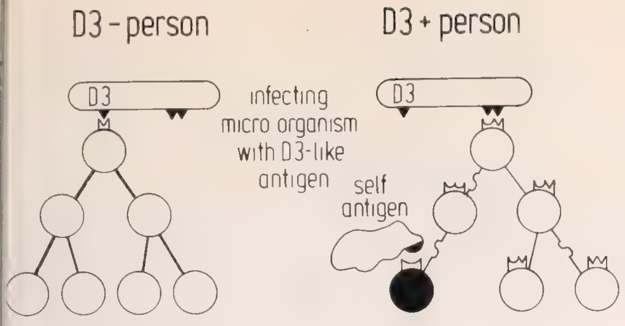
What genes are involved? In the mid-1970s Adams proposed that immunoglobulin V genes were probably responsible for determining the specific inheritance of particular autoimmune diseases, because of the role these genes have in determining antibody specificity (9). This theory could explain the patterns of inheritance of autoimmune diseases but could not readily account for a growing body of information on the role of the major histocompatibility complex (MHC).

In 1964 Lilly and co-workers demonstrated that susceptibility of different strains of mice to infection with Gross virus was determined by a gene associated with the MHC (10). Subsequently, McDevitt and Tyan showed that genes in the MHC could determine the presence or absence of clones reactive with certain synthetic antigens (11). Following on from this, Vladutiu and Rose showed that MHC genes are important in determining susceptibility of mice to induced autoimmune thyroiditis (12). These important findings set the scene for a flood of studies on associations between human diseases and MHC antigens. For some years many people believed that the MHC provided the whole answer to the genetic basis of autoimmune diseases; it was thought that there must be special "disease susceptibility" genes which were in linkage disequilibrium with particular MHC antigen genes (13). However, it now seems probable that it is the histocompatibility genes themselves which play this role, as discussed below.

Combination of Unlinked Dominant Genes. Studies of animal models of autoimmune diseases have provided some very important insights in this whole area. We, like many others, have concentrated efforts on the New Zealand strains of mice, which are strongly genetically predisposed to developing hemolytic anemia (NZB mice) and lupus nephritis (NZB × NZW F_1 hybrids). We found evidence that MHC genes were involved in determining susceptibility to lupus nephritis (8, 14), but the more important observation was that disease susceptibility is also determined by other genes, equally important, on other chromosomes. Susceptibility to lupus nephritis in the NZB × NZW mice is determined by a particular combination of unlinked dominant genes (8). The involvement of non-MHC as well as MHC genes is also observed in induced thyroiditis in mice, and in spontaneous thyroiditis in obese chickens and BUF rats (15).

It is clear that the same involvement is also true in human autoimmune diseases. Three lines of evidence point to this conclusion. The first is the observation that the prevalence of a particular autoimmune disease invariably has been found to be greater in the siblings of probands than in the parents of probands, indicating that in many cases the predisposition is determined by a combination of genes, some from each parent, even though neither parent is affected (8). Second, affected individuals frequently appear in two or more successive generations, suggesting dominance. This has been documented in Graves' disease, pernicious anemia, myasthenia gravis, insulin-dependent diabetes mellitus, and rheumatoid arthritis, among others (8). Third, there is evidence that some of the genes involved are linked not to the MHC but to markers on other chromosomes, including blood group antigens and immunoglobulin allotypic antigens.

Likely Mechanisms. Noting that some of the genes involved in the New Zealand mice appeared to be in the vicinity of minor histocompatibility loci, we realized that there may be a simple answer to the question of what genes are involved. We have proposed that major and minor histocompatibility antigens (including immunoglobulin allotypic antigens, blood group antigens, and the male antigen H-Y) can cause an increase or decrease in likelihood of developing a particular autoimmune disease through the blanks in the immune response repertoire which these antigens must impose during the induction of natural tolerance in the neonate (16). It seems increasingly likely that many autoimmune diseases are triggered by infections with common microorganisms. One likely mechanism by which a particular histocompatibility antigen could increase the likelihood of a particular autoimmune disease is illustrated in Fig. 1. Protective effects of his-



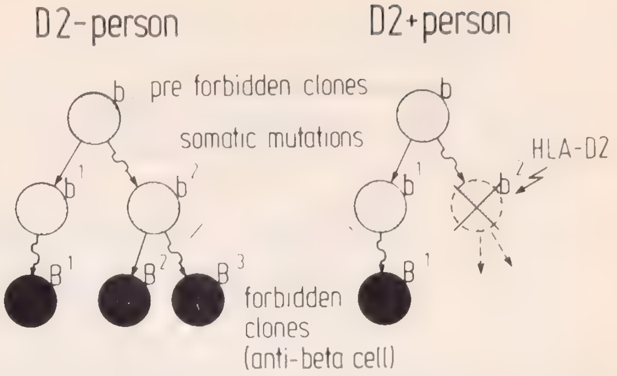


Fig. 1. Left One mechanism by which a histocompatibility antigen such as HLA-D3 could predispose to autoimmune disease triggered by infection. Person lacking D3 is able to mount immune response against D3-like antigen on surface of microorganism. Person with D3 is tolerant of D3 and so must react to some other antigen on microorganism. Sequential somatic mutations affecting paratope (receptor) on surface of lymphocytes reactive with second microbial antigen may result in production of preforbidden clone (stippled lymphocyte) which is capable of diversifying further to produce forbidden clone (black lymphocyte) reactive with particular self-antigen.

Fig. 2. Right Mechanism by which a histocompatibility antigen such as HLA-D2 could have protective effect against disease such as insulin-dependent diabetes. If HLA-D2 cross-reacts with certain precursors of forbidden clones reactive with islet-cell antigen, probability of such islet-cell-reactive clones emerging by somatic mutation will be lessened.

tocompatibility antigens are explicable in terms of their cross-reaction with, and hence deletion of, precursors of pathogenic forbidden clones (Fig. 2).

Evidence for the Forbidden Clone Theory. Burnet's forbidden clone theory postulates that autoimmune diseases occur because of self-reactive clones of lymphocytes arising during life as a result of somatic mutation. Studies of myelomas indicate that a single clone of plasma cells produces antibodies of a single light-chain type—either κ or λ. It follows logically from this that in a disease such as Graves' disease, if the initiating event is a mutation in a single lymphocyte, from which a number of closely related clones develop by subsequent mutations, in most cases all the autoantibodies in an individual patient would be expected to be of a single light-chain type—either κ or λ. The first three studies of this question found that this was not so (17–19). However, the method used in each case was immunoprecipitation, and we have observed that nonspecific coprecipitation occurs with this technique. Recently, Zakarija reexamined this question by affinity chromatography using heterologous antisera immobilized on columns and found that virtually all of the thyroid-stimulating autoantibodies (TSab) in any one patient were of a single light-chain type (20).

We have carried out affinity chromatography on several more sera using monoclonal anti-κ and anti-λ antibodies (unpublished results), and the Table shows the results of a typical experiment. In all, we have studied serum from 11 patients and have used an extremely sensitive enzyme-linked immunosorbent assay (ELISA) to detect κ or λ determinants on any residual antibodies after depletion. All 11 serum samples had TSab of a single light-chain type, and 10 of these were λ. In our view this supports the forbidden clone theory, in that the data are compatible with the proposition that the TSab of an individual patient have arisen from a single precursor by a series of sequential mutations. If our data are pooled with those of Zakarija, 17 of 19 patients produced TSab of λ light-chain type. This suggests that cells expressing λ V genes are more likely to undergo the particular mutations needed to produce TSab specificity than are those expressing κ V genes. Furthermore, it has reawakened our interest in the possibility that immunoglobulin light-chain V genes may be genetic determinants for autoimmune diseases in humans (9). We hope that family studies in various diseases can resolve this question.

The evidence from Volpé and co-workers (21), indicating that normal people have specific clones of suppressor cells that are absent from people

TABLE

Confinement of Long-Acting Thyroid-Stimulator (LATS) Activity to Immunoglobulins with λ Light Chains in Serum from Patient Ar, with Graves' Disease

Preparation assayed	LATS response*	
Unfractionated serum	1540 ± 267	$p < 0.001$
Serum after anti-λ column passage	−26 ± 7	NS
Serum after anti-κ column passage	1302 ± 552	$p < 0.001$
Eluate from anti-λ column	582 ± 187	$p < 0.001$
Eluate from anti-κ column	−2 ± 10	

* Percent rise in blood ^{125}I in assay mice, ± standard error of mean.

with autoimmune disease of the thyroid and pancreatic islets, raises the possibility that only people who have a congenital absence of such clones from their repertoire are at risk of developing autoimmune diseases by somatic mutation of V genes.

Summary

In humans, a genetic predisposition underlies autoimmune diseases, but the expression of such diseases requires environmental influences and the occurrence of random events such as somatic mutations. Animal models of autoimmune diseases have provided important insights into the genetic mechanisms underlying this predisposition. It is apparent that multiple unlinked genes are responsible, including the MHC genes. Other genes likely to be involved are those that influence the immune response repertoire, including antibody structural genes and genes for minor histocompatibility antigens.

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HLA Class II Antigens and the Human Thyroid Cell: A Review

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It is surprising how long it has taken endocrinologists to realize that the main stage for autoimmune thyroid disease is the thyroid itself. We seem to have been mesmerized by the presence of peripheral thyroid antibodies and relatively weak group HLA associations. However, studies on intrathyroidal lymphocyte function have clearly demonstrated that the thyroid is the place where the action occurs (1–3). Such studies have assumed added importance with the finding of HLA class II antigens on the surface of the thyroid cell (4) and the role of these antigens within the immune system (5). This paper summarizes what we mean by "importance" and includes a basic introduction to the class II antigens. It is critical to realize that HLA antigens are not present on the surface of cells for the convenience of tissue-typing laboratories. Such antigens serve important physiological roles and we believe that they may be important in the etiology and maintenance of autoimmune thyroid disease.

Molecular Biology of HLA Class II Antigens

Human HLA-D region (class II) antigens, like their murine H-2 I region counterparts, have particular importance within the immune system for antigen presentation and recognition of self (6). Class II histocompatibility antigens are constitutively expressed on the surface of B cells, activated T cells, and macrophages (7, 8) as well as a variety of nonlymphoid cells (see section on Class II Antigens in Nonimmune Cells, below). The HLA class II antigen molecule is a dimer, consisting of single alpha (34 kd) and beta (28 kd) glycopeptide chains, which are linked noncovalently within the plasma membrane and are associated intracellularly with an invariant (33 kd) glycopeptide (9). The alpha and beta chains are encoded within the HLA-D region of the human major histocompatibility complex (MHC) region located on the short arm of chromosome 6. The invariant chain is encoded outside the human MHC, on chromosome 5 (10), and is believed to be involved in the assembly and/or transport of the class II antigens.

The HLA-D class II alpha and beta chains (Fig. 1) (11) consist at their amino termini of two external domains (alpha 1 and alpha 2, beta 1 and beta 2), each of which contains approximately 90 amino acids and shares structural homology with the immunoglobulin molecule constant (C) region domains (12). The two membrane-proximal domains, alpha 2 and beta 2, have significant amino acid sequence conservation compared to the membrane-distal domains, alpha 1 and beta 1 (13).

Polymorphic Genetic Variation in HLA-D Region. The HLA-D region has been subdivided into three major subregions, DP, DQ, and DR, which encode structurally similar but immunologically distinct class II antigens (Fig. 2). The genetic variation within the human population at the HLA-D region may be attributable in large part to the differences in amino acid sequence within the first external domains of the alpha and beta chains.

The alpha chain has been reported to have limited polymorphism and has been termed "invariant," whereas the beta chain appears to be highly polymorphic (14). To date, six HLA-D re-

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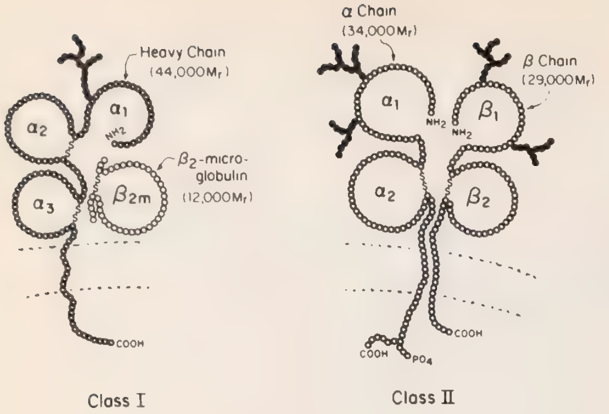

Heavy Chain
(44,000Mr)
α1
α2
α3
NH2
β2-micro-
globulin
(12,000Mr)
β2m
COOH
Class I
α Chain
(34,000Mr)
β Chain
(29,000Mr)
α1
β1
NH2
NH2
α2
β2
COOH
COOH
PO4
Class II

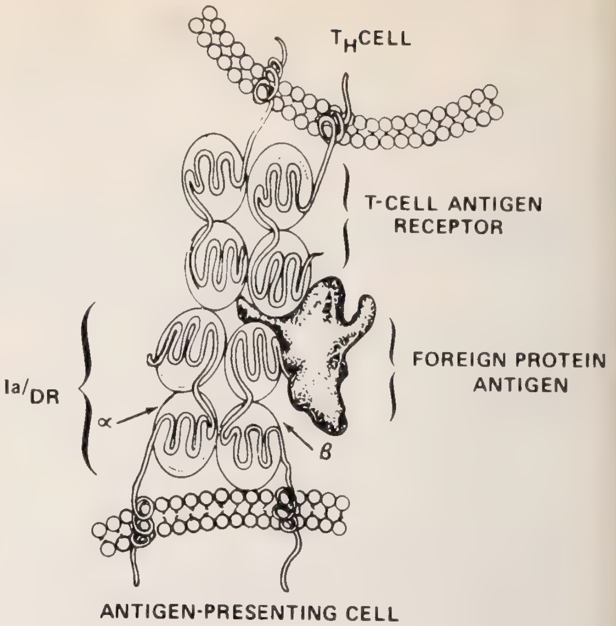

THCELL
T-CELL ANTIGEN
RECEPTOR
FOREIGN PROTEIN
ANTIGEN
Ia/DR
α
β
ANTIGEN-PRESENTING CELL

Fig. 1. Above Structure of class II MHC. Reprinted, with permission, from Gjerset et al (11), 505.

Fig. 3. Right Possible method of combination between I-A (DR) antigen and foreign antigen. Exact position of foreign or self-antigen in relation to I-A (DR) molecule remains uncertain. Reprinted, with permission, from McDevitt (23).

gion alpha chains per haploid genome have been described (15). Two of the alpha chain genes are encoded within the DP subregion, two are closely linked to DQ, and one maps within the DR subregion. The remaining alpha chain gene has not yet been definitively mapped within the D region. The degree of expression of these six alpha genes is not yet known; however, the existence of at least one alpha chain "pseudogene" has been suggested (16). It appears that a minimum of three alpha chain genes must be functional (transcriptionally and translationally active) to account for the existence of three distinct HLA-D region products: DP, DQ, and DR.

The genetics of the HLA-D beta chain is less clear, but seven beta chain genes have so far been discovered within the human haploid genome (17), and they display a high degree of genetic polymorphism. The DQ and DP subregions each encode at least two, and the DR region at least three distinct beta chain genes.

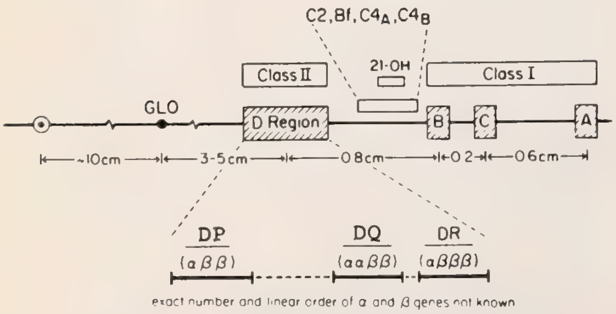

C2, Bf, C4A, C4B
Class II
21-OH
Class I
GLO
D Region
B
C
A
~10cm
3-5cm
0.8cm
0.2
0.6cm
DP
(αββ)
DQ
(ααββ)
DR
(αβββ)
exact number and linear order of α and β genes not known

Fig. 2. Plan of HLA-D region. Reprinted, with permission, from Gjersat et al (11), p. 504.

Genetic mapping of the HLA-DP region has indicated an alpha/beta/alpha/beta gene arrangement, where the alpha chain genes are transcriptionally oriented in the opposite direction from the two beta chain genes (16, 18). This gene arrangement is believed to have arisen from a duplication event involving the "proto-alpha/beta" gene sequence with subsequent genetic divergence.

The HLA-DR subregion is reported to be in strong genetic linkage disequilibrium with DQ (15). Therefore, certain DR alleles are found associated in high frequency with certain DQ alleles. The DP region is less closely linked to either DQ or DR and is the most centromeric of the three D subregions on chromosome 6.

The HLA-D region gene family appears, then, to be composed of polymorphic genetic variants closely linked on a single chromosome, and to have arisen via multiple duplication events.

Function of Class II Antigens in the Immune System

Vertebrates manifest wide intraspecies as well as interspecies differences in their responses to antigenic stimuli, and such variation in immune responses has been linked to the major histocompatibility complex (MHC). In humans, class II immune-response genes are encoded within the D region of the HLA locus, the human equivalent of the MHC. However, until recently, the murine model had been explored in greater detail than humans. In mice the MHC is referred to as the H-2 locus located on chromosome 17. This region contains the immune response (Ir) genes which specifically map within the H-2 I region. The H-2 I region has also been shown to encode immune-response-associated (I-A and I-E) antigens, MHC class II antigens, which have allowed the correlation between structure and function of immune response genes to be explored (19). Class II antigen-bearing cells in animals and humans can be detected by a variety of methods:

Serology: cytotoxicity; immunofluorescence; immunoperoxidase.

Immunochemistry: sodium dodecyl sulfate (SDS) gel electrophoresis; tryptic peptide mapping; sequencing of purified antigen.

Molecular biology: DNA analysis of α, β, or invariant chains; mRNA analysis of α, β, or invariant chains.

Anti-class II sera have the potential to inhibit immune responses in vitro and in vivo, including autoimmune reactions of many types (20–22). Indeed, this ability was used as strong evidence in favor of murine I-a antigens begin Ir gene products. What, then, is the physiological role for such molecules? Class II MHC antigens were found initially on the surfaces of B cells, macrophages, dendritic cells, and activated T cells (7, 8). The particular function of monocyte/macrophage-associated class II antigens is now beginning to be understood. Large protein antigens are "processed" by macrophages possessing surface class II molecules, resulting in molecular changes or fragmentation and an association between the class II molecule and antigen on the cell surface (Fig. 3) (23). Such interactions cause specific T cell subset responses to antigen, followed by T cell proliferation and production of soluble messengers (lymphokines). Lymphokines stimulate certain B cell as well as additional T cell clones, resulting in an immune response to the antigen (or self-antigen).

It is not entirely clear how the macrophage is directed to process certain antigens and not others. Indeed, such processing may be nonspecific (24), and the specificity of the reaction may lie at the level of T cell receptors for the antigen/class II antigen complex (25).

On the basis of this model, class II antigen appears to be an important molecule, mediating antigen recognition between interacting cells in the immune network. Indeed, class II antigens have such a critical role that many immune responses do not occur in the absence of these antigens. Such responses are termed MHC-restricted (20).

MHC class II antigens also appear as cell surface markers during hematopoietic development and may play a role in cell-cell interactions during cellular differentiation (26).

Class II Antigens in Nonimmune Cells

Bone-Marrow-Derived Cells. In recent years it has become apparent that the expression of MHC class II antigens is not restricted to the immune system. It was not surprising to find such antigens on dendritic cells, since these cells may function similarly to, and be derived from, the monocyte/macrophage line (27). Hence, epidermal Langerhans cells may be class II-positive in animals and humans and are important in skin allograft immunity (26). Many disorders of the central nervous system, including multiple sclerosis, have been associated with immune or autoimmune phenomena (28). Class II-positive cells, although they represent a small proportion of the total cell number, have been identified in both animal and human brains (29). Evidence suggests again that such cells are bone-marrow-derived and may represent dendritic-like antigen-presenting cells (26). A similar series of investigations have shown that the phagocytic Kupffer cells in liver express class II molecules and perform immune regulatory roles (30). In the lung, class II positivity appears to be confined to the alveolar macrophages (31). In addition, class II antigens are expressed in a variety of hematopoietically derived tumors, including many leukemias (32).

Hence, monocyte/macrophage/dendritic-like cells appear to express MHC class II antigen throughout the body, and such molecules may serve important immune surveillance functions.

"Aberrancy" in Tissues. MHC class II antigens can also be "aberrantly" expressed in a variety of tissues. Often such tissues contain immune infiltration with local release of interferons, potent inducers of class II antigens (33). For example, bile duct epithelium in primary biliary cirrhosis (34), renal tubular cells in renal allografts (35), pancreatic acinar cells of diabetic animals and humans (36), human trophoblast cells (37), hair follicles (38), colonic epithelium (39), and thyroid cells all express class II antigens, as discussed in detail below. In addition, a variety of tumors derived from cells which do not usually express class II antigens have been reported to be class II-positive, including melanomas (40) and breast and lung carcinomata (41). Indeed, it appears that many cell types have the capacity to express class II antigens under appropriate circumstances (42). Tissue-specific factors may regulate, perhaps via gene promoter sequences, the restriction of class II antigen expression. The term "aberrant class II expression" has, therefore, to be carefully defined, since such expression may be appropriate to the environment in which the cell finds itself.

HLA-DR Antigen in Thyroid Cells

Early studies indicated that although thyroid tissue is positive for the occurrence of MHC class I antigens, in fact class II antigens occurred on only a small subpopulation of thyroid tissue cells, the dendritic cells (43). Such a distribution of MHC antigen has been observed in many other tissues. However, the basal area of murine thyroid cells was found to contain small quantities of I-A antigen (44). It was also possible to induce class II antigen in over 90% of normal human thyroid cells, employing the same approach used for lymphocyte stimulation: using mitogens (5, 45) or gamma interferon (46) (Fig. 4). Furthermore, it was observed that thyroid tissue from patients with autoimmune thyroid disease (both Graves' and Hashimoto's diseases) "spontaneously" exhibited DR antigen on histologic sections of thyroid tissue (47), although principally in the region of the most intense immune infiltrate (48). Such expression of DR antigen was lost with prolonged in vitro culture or after transfer of intact tissue to nude mice (49). These data suggested that the human thyroid cell responds secondarily to immune signaling by producing HLA-DR antigen. We have also observed HLA-DQ antigen in normal thyroid cell cultures, though at lower levels than HLA-DR [mean ± standard deviation, 49.9 ± 16.4% of lectin-stimulated normal cells; see (5)]. The importance of HLA-DQ in the human immune response remains to be further evaluated, but it may be closely linked with DR antigen function (50).

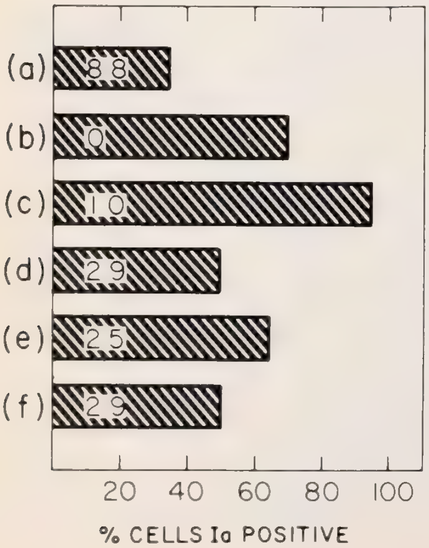


Fig. 4. Induction of I-A (DR) antigen in human thyroid cells. Experiments demonstrating I-A (DR) antigen induction in normal human thyroid cells with leukoagglutinin (10 µg/ml). Basal mean percentage of positive cells without lectin is shown for each experiment.

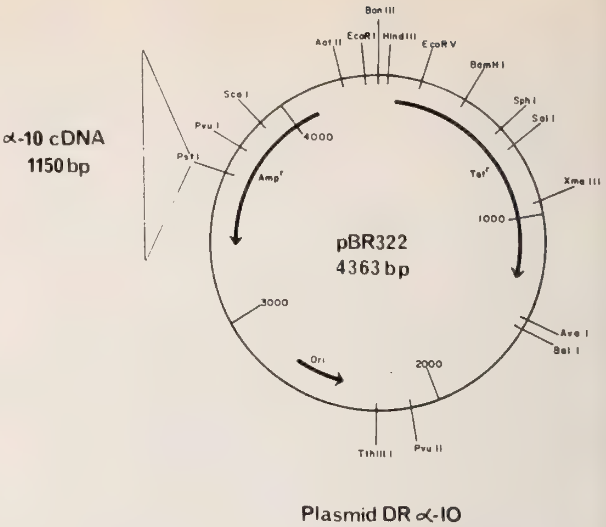
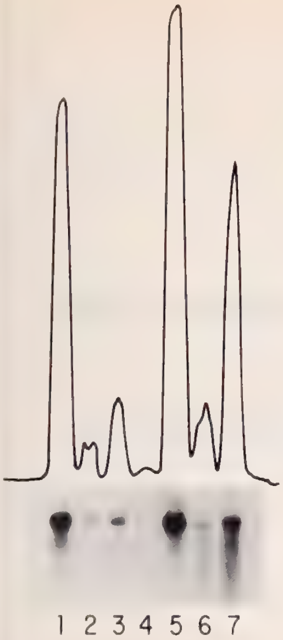


Fig. 5. Complementary DNA probe for HLA-DR alpha chain. Reprinted, with permission, from Wake et al (51).

Recently, we used a ^{32}P-labeled complementary DNA probe encoding the HLA-DR alpha chain and cloned from the human Raji B lymphoblastoid cell line (Fig. 5) (51). There was a single HLA-DR alpha-chain-specific messenger RNA species, less than 2 kb in size by Northern blot analysis, in total cellular RNA prepared from a variety of human thyroid tissues (Fig. 6) (52). Dot blot analyses of cellular RNA have shown that Graves' hyperthyroid tissue contains high levels of DR alpha-chain-specific mRNA, far in excess of any possible contribution from lymphocytic infiltration. In addition, studies with human thyroid cell monolayer cultures, depleted of lymphoid cells and monocyte/macrophages as demonstrated using FITC-labeled murine monoclonal antibodies, have confirmed the existence of transcriptionally active HLA-DR alpha chain gene(s) in human thyrocytes. Cytoplasmic slot blot analyses of extracts prepared from 5-day thyroid cell monolayers indicated that normal thyroid cells contain low but detectable levels of alpha-chain-specific mRNA. Furthermore, HLA-DR alpha chain gene in normal human thyroid cells exposed to mitogen may be induced to levels comparable to those found in Graves' tissue (Fig. 7, lane A, row 8).

These data demonstrate that normal thyroid cells contain detectable levels of HLA-DR-specific mRNA and that such cells may contain low levels of DR antigen not normally detected by immunofluorescence techniques. In addition, the response of thyroid cells to mitogen and/or gamma interferon is variable and may be dependent on



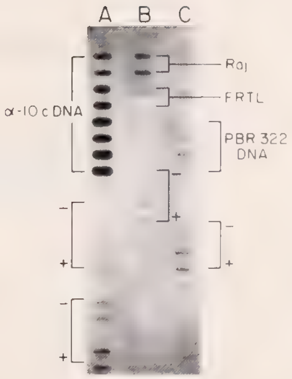


Fig. 6. Left Northern blot analysis of HLA-DR alpha chain transcripts. Densitometric tracing of relative signal intensity is depicted above Northern blot analysis of DR alpha transcripts in total cellular RNA isolated from human tissues and Raji cell line and probed with DR alpha chain cDNA (52). Sources of RNA samples (25 μg per lane): lane 1, Graves' thyroid tissue (patient A); lane 2, thyroid adenoma tissue; lane 3, Graves' thyroid tissue (patient B); lane 4, testis tissue; lane 5, Raji cell line; lane 6, normal thyroid tissue; lane 7, Graves' thyroid tissue (patient C).

Fig. 7. Right Cytoplasmic slot blot analysis of HLA-DR alpha chain transcripts in human thyroid cells. Normal human thyrocyte cultures were established from thyroid tissue obtained at surgery (5) and grown with (+) or without (−) lectin (leukoagglutinin, 10 μg/ml) for 5 days. Cytoplasmic extracts to be used in DR alpha chain transcript analysis were prepared by method of White and Bancroft (57). Briefly, cells were lysed in presence of 1% Nonidet P 40 and nuclei were removed by centrifugation (13,000 × g, 2.5 min) in Fisher model 235B centrifuge. Cytoplasmic RNA in supernatants was denatured at 60°C for 15 min in 7% formaldehyde and applied to Gene Screen filters (New England Nuclear), using Minifold II slot blot apparatus (Schleicher & Schuell) Prehybridization, hybridization, and autoradiography were performed as described elsewhere (59). Cytoplasmic extracts or known amounts of DNA standards were applied in duplicate slots as follows. (Lane A) DR alpha cDNA: rows 1–4, 10, 25, 50, 100 pg, respectively; normal thyroid cell: patient D (1×10^5 cells per slot); row 5, control; row 6, +lectin; patient E (2×10^5 cells per slot); row 7, control; row 8, +lectin. (Lane B) (3.5×10^5 cells per slot) Raji cell line: row 1, Fisher rat thyroid cell line; row 2, normal thyroid cell: patient F; row 4, control; row 5, +lectin. (Lane C) Plasmid pBR322 DNA; rows 1–4, 10, 25, 50, 100 pg, respectively; normal thyroid cell: patient G (7×10^4 cells per slot); row 5, control; row 6, +lectin.

HLA-DR with respect to the ease and degree of DR antigen induction and the effects of such expression on the immune system within the thyroid (52).

Role in Thyroid. To investigate the role of class II antigen expression by the human thyroid cell in its relationship with the immune system, we have employed experimental in vitro coculture techniques. Human thyroid cell monolayers were cocultured with autologous lymphocytes over a 5-day period. Studies were performed on thyroid cells (referred to as "normal") from individuals coming to surgery for benign follicular adenomata and on thyroid cells from patients with hyperthyroid Graves' disease who were undergoing subtotal thyroidectomy.

No evidence of T cell activation occurred when 5-day normal human thyroid cells were cocultured with autologous T cells (53). However, T cells cocultured with normal autologous thyroid cells, expressing high levels of DR antigen after preincubation with mitogen (leukoagglutinin) or gamma interferon, were stimulated to proliferate (Table 1) (5). Results of earlier experiments with Graves' thyroid cells in the absence of mitogen preincubation, in coculture with autologous T cells, demonstrated a small but significant degree of T cell activation (Table 2), which was enhanced by the presence of T cell growth factor (interleukin-2) (Fig. 8) (53). These data suggested that DR antigen, spontaneously expressed by Graves' thyroid cells, participated in the initiation of T cell activation. We hypothesized that class II antigens amplify the intrathyroidal T lymphocyte

TABLE 1

T Cell Proliferation (Autosensitization) on Coculture of Normal Human Thyroid Cells and T (E^-) Cells

Experiment*	Thyroid cells (a)	E^- cells (b)	E^-/thyroid (c)	p value (a) + (b) versus (c)
1	2,067 ± 426	352 ± 62	1,352 ± 377	NS†
2	1,919 ± 217	144 ± 32	1,329 ± 123	NS
3	1,271 ± 239	108 ± 5	890 ± 170	NS

* 50,000 thyroid cells were cultured with 500,000 T cells. Data (counts per minute) are expressed as mean ± standard error for the group. Control mitogen and T cells only gave a mean ± standard error of 75,855 ± 3,646 cpm.

† NS, not significant.

Adapted from Davies TF et al (53).

TABLE 2

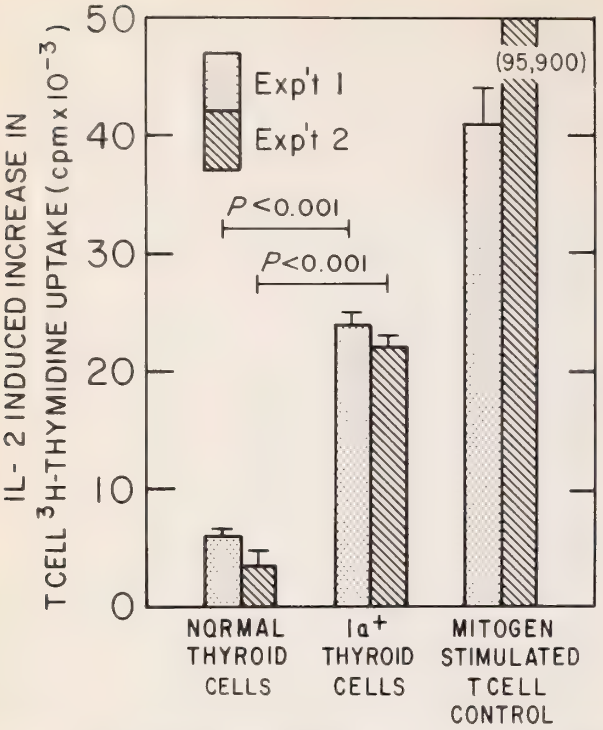
T Cell Proliferation (Autosensitization) on Coculture of Graves' Thyroid Cells and T (E^+) Cells

Patient	Thyroid cells (a)	E^- cells (b)	E^-/thyroid (c)	p value (a) + (b) versus (c)
1	1,890 ± 250	1,020 ± 86	4,630 ± 294	<0.05
2	832 ± 110	499 ± 62	2,064 ± 110	<0.05
3	243 ± 80	213 ± 21	1,571 ± 170	<0.05
4	748 ± 84	396 ± 72	708 ± 68	NS†

* Details are as described for Table 1 and are the mean results for triplicate determinations (data as mean ± standard error counts per minute from Fig. 3). Control mitogen and T cells only gave a mean ± standard error of 65,736 ± 7,815 cpm.

† NS, not significant.

Adapted from Davies TF et al (53).



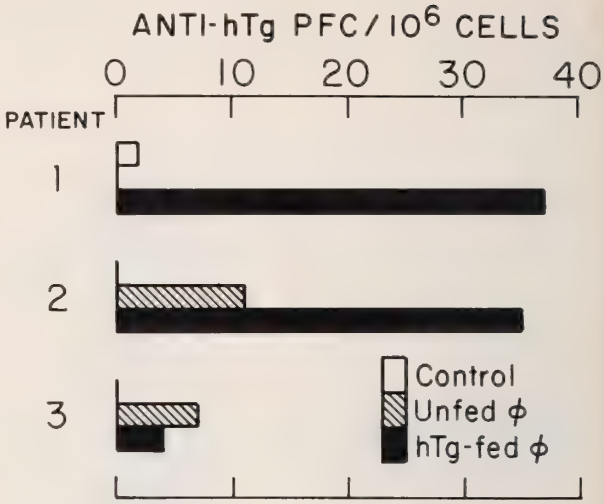


Fig. 8. Left Influence of interleukin-2 (IL-2) on T cell proliferation. Autologous thyroid and T cells were cocultured for 5 days without IL-2, then for 5 days with IL-2 (10%) prior to measurement of [^{3}H]thymidine uptake. Data expressed as counts per minute of [^{3}H]thymidine (mean ± standard error) in triplicate wells. Mitogen-stimulated T-cell-only control is shown for both experiments illustrated.

Fig. 9. Right Macrophage presentation of human thyroglobulin (hTg) to T-B cells of patients with autoimmune thyroiditis. Autologous macrophages were prefed with or without hTg and then cocultured with B-T cells (58). Specific anti-hTg plaque-forming cells (PFC) were then measured. Note lack of response in experiment 3. Anti-hTg PFC were not observed for two other patients investigated in this series of experiments. Bars indicate standard errors.

population in persons with Graves' hyperthyroidism, and that this phenomenon is secondary to the local release of gamma interferon. Similar results were reported for human malignant melanoma cells, which express DR antigen on their cell surface, compared to melanocytes, which are DR-negative (40).

Hence, class II-mediated activation of many intrathyroidal T cells would include appropriate thyroid-responsive T cell subsets, which may lead to the expansion of thyroid antibody-secreting B cell clones, if they are present, and result in the initiation or progression of autoimmune thyroid disease. However, this amplification phenomenon is not disease-specific, since we have induced T cell amplification in cocultures of DR-positive normal thyroid cells and T cells. Early data indicate that such T cells are primarily helper cells. Hence such observations also explain the decreased influence of suppressor T cells in the intrathyroidal environment and do not require hypothesizing a defect in suppressor T cells (54, 55).

The etiology of the initial T cell stimulation within the thyroid gland of patients with autoimmune thyroid disease and the subsequent release of gamma interferon and increase in DR antigens remains unclear. We believe it is possible that thyroid-cell DR antigen may not only act as an immune response amplifier, but may also participate directly in presenting thyroid antigen to the immune system. Antigen presentation by the thyroid cell may explain the initiation of T cell stimulation in individuals who develop thyroid-reactive T cells. Such thyroidal presentation would imply bypassing the classical macrophage processing mechanisms. In this regard, we and other workers have demonstrated that autoantigens are processed in the same way as foreign antigens. We found that autologous macrophages stimulate thyroid autoantibody secretion after thyroglobulin processing (Fig. 9), and therefore it is possible that the thyroid cell itself is equally or better suited to function in the presentation of thyroid-specific antigens to the immune system. Such action by the thyroid cell is supported by evidence for thyroid cell presentation of previously processed viral antigen (56), consistent with the function of cells which express class II antigens. However, the presentation of thyroid-specific antigen remains to be more fully investigated. We have been able to induce secretion of antibodies against human thyroglobulin by exposure of T-B cells from patients with Hashimoto's disease to thyroid cell monolayers. These results imply that thyroid cells participate not only in T cell proliferation but also in thyroid-specific antigen presentation.

Summary

A rapidly expanding literature has demonstrated the association between certain HLA subtypes and autoimmune thyroid disease. The observation of HLA class II antigen expression by the human thyroid suggests that the HLA associations observed in population studies may have mechanistic explanations at the level of the thyroid cell itself. Evidence to date convincingly shows HLA-DR antigen in both normal and abnormal thyroid cells, and enhanced HLA-DR in patients with autoimmune thyroid disease. Furthermore, it appears that thyroid cell HLA-DR antigen may participate in T cell activation and may also be involved in presentation of antigen to the immune system. Understanding the relationship between these immune interactions and the development of autoimmune thyroid disease may lead to a fundamental change in our understanding of the etiology of autoimmunity.

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Family Studies in Autoimmune Thyroid Disease

Sheila H. Roman, M.D.

The role of genetic factors in the development of autoimmune thyroid disease has been inferred from clinical and epidemiologic observations, including the occurrence of familial clustering of these diseases, the tendency of probands and their first-degree relatives to develop autoimmune phenomena of multiple organs, the higher concordance rates for disease in monozygotic than in dizygotic twins, and the association of these diseases with the HLA gene complex.

Observations on Familial Aggregation. It is estimated from studies in several countries that siblings and offspring of persons with autoimmune thyroid disease are over ten times more likely than other persons to develop thyroid disease (1–3). Antithyroid and other organ-specific autoantibodies are increased in first-degree relatives of patients with thyroid disease (4–13). Moreover, carefully controlled sero-epidemiologic studies have demonstrated that the predisposition to form autoantibodies to thyroid antigens is familial, even when no thyroid disease is clinically evident (5). Also documented in families of patients with autoimmune thyroid disease are increased proportions of individuals with goiter, abnormal thyroid function tests (4, 14–16), and altered cell-mediated immunity (17).

Such familial aggregations support, but do not conclusively substantiate, a genetic basis for autoimmunity. To further support the role of genetic factors in the development of autoimmune thyroid disease, increased concordance rates for Graves' disease have been found in monozygotic as compared to dizygotic twins of the same sex (18–21). Since the concordance rate for identical twins is only about 50%, these surveys indicate that environmental factors may be important. Graves' disease and Hashimoto's thyroiditis frequently occur in the same family, and even in monozygotic twins—one twin having Graves' disease, the other having Hashimoto's thyroiditis (22).

Genotyping. Both Graves' disease and Hashimoto's thyroiditis have been associated with the major histocompatibility complex (MHC), which has become accepted as the main immunoregulatory gene complex. The MHC consists of three classes of human leukocyte antigen (HLA): A, B, and C (class I); DR, DQ, and DP (class II); and the linked genes for the complement components, Bf (properdin factor B), C2, and C4 (class III). There is a pronounced linkage disequilibrium between these various loci, some genes occurring much more frequently in the same individual than would be expected from the frequency of each gene alone.

Graves' disease was initially associated with B8 in caucasoids (4, 9, 23–28). Later the disease was found to be more strongly associated with DR3, which in turn was in linkage disequilibrium with B8 (29–30). In other populations with different distributions of HLA haplotypes, there are different HLA associations—as, for example, in Japan, where B8 is relatively rare and Graves' disease is associated with Dw12 (31). Genetic heterogeneity for DR association has been reported for Hashimoto's thyroiditis. Atrophic thyroiditis is associated with DR3 (32) and secondarily with B8 (33); goitrous thyroiditis is associated with DR5 (34–36).

The relative risk for development of autoimmune thyroid disease attached to any of these antigens is relatively low, and 30%–50% of patients

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with disease do not express an associated HLA antigen. However, HLA-B8 and DR3 have been associated with several other autoimmune diseases (37) and also with the in vitro demonstration of immunoregulatory abnormalities in normal persons (38–40). This implies either a broad immunopotentiating influence for these antigens interacting with other genetic markers and environmental factors, or that the disease-susceptibility gene may be in further linkage disequilibrium.

Genes that specify phenotypic markers of immunoglobulins have also been correlated with autoimmune diseases (41). One of these phenotypes is a polymorphic serologic marker on the heavy chain of immunoglobulins termed Gm. Controversy exists over whether specific Gm allotypes are, or are not, associated with autoimmune thyroid disease (41–43). One study has also shown an association with light-chain immunoglobulin markers (Km) in six highly selected families affected with both Graves' disease and Type 1 diabetes (44), but this finding has been disputed (45–46).

Prospective Studies and Family Analysis. There are few prospective studies of individuals at risk for autoimmune thyroid disease.

Carey et al (14) investigated 153 children of patients with Graves' disease and found a thyroid-related abnormality in 55 children (36%). Abnormalities included Graves' disease treated by partial thyroidectomy in two children, physically abnormal thyroid, and serologic abnormalities, including high or low T4 or T3, abnormal thyroglobulin, elevated TSH, or the presence of thyroid autoantibodies. Four children were considered to have definite evidence of Hashimoto's thyroiditis and two children had TSH receptor antibody associated with elevated T3 or T4. Of the 153 children, 59 were followed up after one year; 9 (15%) who had been normal had acquired an abnormality, 13 (22%) had retained the original abnormality, 8 (14%) had acquired another abnormality, 13 (22%) had lost the original abnormality, one had developed Hashimoto's thyroiditis, and one had developed Graves' disease.

Tamai et al (16) reported on thyroid function in 206 clinically and chemically euthyroid individuals who had at least one first-degree relative with Graves' disease. Of the 206, 56 (27%) showed abnormal responses to TRH and 8/117 (7%) tested were T3 nonsuppressible. Among 69 of the 206 followed up (6 months–5 years), 3 developed Graves' disease and 2 developed hypothyroidism.

Such studies provide information on the premonitory phase of autoimmune thyroid disease and the incidence of disease development in persons at risk, but do not identify the genetic factors and immunopathologic mechanisms predictive of disease development. Uno et al (47), in their study of 15 Japanese families having more than two first-degree relatives with Graves' disease, found that 16 out of 17 affected sib pairs shared a particular HLA and IgG-linked gene combination. In a further study investigating 37 such families (48), this group again found that of 96 members with Graves' disease and 14 members with Hashimoto's thyroiditis, all but one had their family's disease-associated haplotypes of both HLA and Gm. Although these findings could imply the existence of two genes controlling disease susceptibility, 25% of family members with the disease-associated haplotypes of both HLA and Gm were euthyroid, and specific HLA and Gm disease-associated haplotypes could not be identified within the whole population.

Torfs et al (49) used the LIPED computer program to calculate the likelihood of dominant and recessive models of inheritance of autoimmune thyroid disease in families in which the proband had either Graves' disease, Hashimoto's thyroiditis, rheumatoid arthritis, Type I diabetes, or systemic lupus erythematosus. The preliminary conclusion was for dominant inheritance of thyroid disease. In contrast, Stenszky et al, analyzing by the sibling pair method, found a distribution of HLA haplotypes associated with Graves' disease consistent with recessive inheritance (50). Such data suggest that we may be able to genetically identify susceptible individuals within families but do not give us any information on the immunologic mechanisms involved.

Immunogenetics of Thyroid Autoimmunity in Type I Diabetic Families. Thyroid autoantibodies are increased in Type I diabetics and their first-degree relatives (51). There are also MHC associations in common, since Type I diabetes, Graves' disease, and atrophic Hashimoto's thyroiditis are all associated with an increased prevalence of DR3. I have been involved in a collaborative study which has afforded us the opportunity of measuring thyroid autoantibodies in 84 HLA-typed families in which a proband child has Type I diabetes. Using sensitive enzyme-linked immunosorbent assay (ELISA) techniques (52), we found detectable thyroid autoantibodies in 49% of our study population and a significant relationship between the presence of thyroid microsomal antibody and HLA-DR5 (53), the antigen previously associated with goitrous autoimmune thyroiditis.

A previous study of Type I diabetic families also found thyroid autoantibodies to occur independent of the HLA-linked susceptibility to diabetes (54). Yet non-HLA factors were also important in our study, since the DR5 association was true only for male subjects, and the majority of antibody-positive individuals did not possess DR5. In preliminary data from the further investigation of Gm allotypes in these families, we could identify neither a Gm phenotype significantly associated with thyroid autoantibody presence nor HLA and Gm interactions jointly affecting the detectability of thyroid autoantibodies in these selected families (55). We conclude that the presence of thyroid autoantibodies is the result of multiple genetic and nongenetic factors, which may act independently or interrelatedly in ways which may change their individual influence on autoantibody occurrence.

Hypothesis: Two Genetic Defects. Many questions remain on genetic interactions and resultant immune responsiveness in persons at risk for autoimmune thyroid disease. The literature nowhere provides information on specific immunogenetic mechanisms that may lead to the development of clinical disease. Yet the observations reviewed here are consistent with the hypothesis that expressed autoimmunity results from at least two genetically determined defects. One is a rather diffuse disorder in immunoregulation that allows individuals under the right stimulation to be autoreactive and produce autoantibodies to self-antigens. When this is coupled with a second more specific susceptibility to organ-directed B and/or T cell activation, a particular autoimmune disease is clinically expressed. The genetic control resulting in these autoimmune events is potentially complex, and there are multiple possibilities for the cause of immune dysfunction, even within groups of patients who clinically appear to have the same disease. Theoretically the use of these immunogenetic determinants and a more thorough understanding of their role in the control of immune events should allow us to predict the clinical expression of autoimmune thyroid disease.

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Thyroid Autoimmune Disease
Part 2:
Mechanisms

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Mechanisms of Thyroid Autoimmune Disease: Introduction

ROBERT VOLPE, M.D.

The four papers in this section concentrate on mechanisms involved in the complex problem of autoimmune thyroid disease and present the current state of our knowledge.

Human Thyroid Antibody Regulation. Dr. Sandra McLachlan deals with in vitro antibody control and regulation in humans. She and others have shown that lymphocytes from different lymphoid organs differ in their ability to secrete thyroid autoantibodies spontaneously or in response to pokeweed mitogen.

In the peripheral blood compartment, in most instances of autoimmune thyroid disease, the B lymphocytes are not active producers but rather are memory cells. They can, however, be stimulated to produce autoantibody by mitogenic stimulation. In a few instances, peripheral blood lymphocytes taken from patients with Hashimoto's thyroiditis do produce thyroid autoantibodies actively and spontaneously and can be stimulated by antigen, but this active production is emphatically not the rule.

McLachlan shows that thymic tissue does not usually contain plasma cells that secrete thyroid autoantibody, in contrast to the situation in myasthenia gravis. Lymph nodes draining the thymus are intermediate between the thyroid and the blood, in that they sometimes contain memory cells (activatable by pokeweed mitogen and T lymphocytes) and sometimes contain cells actively producing thyroid autoantibodies.

Thyroid-derived lymphocytes from patients with Hashimoto's thyroiditis and Graves' disease are, however, active, spontaneous producers of thyroid autoantibodies in the absence of in vitro mitogenic or antigenic stimulation. This has been observed in my own laboratory by Beneveniste et al (*Clinical and Experimental Immunology* 1985; 61:274). Thus, the thyroid gland is certainly the most important source of thyroid autoantibody synthesis in human autoimmune thyroid disease.

Effect of Antithyroid Drugs. In her paper, Dr. McLachlan further shows the effects of antithyroid drugs on the ability of intrathyroidal lymphocytes to grow in tissue culture. Even when antithyroid drugs have been discontinued at least ten days before surgery, there is still a marked effect on the ability of the intrathyroidal lymphocytes to grow. Since I understand that thyroid tissue is completely cleared of the antithyroid drugs a few days after the patient stops taking them and long before the surgery itself, the question arises, precisely how does the antithyroid drug act on the microenvironment of the thyroid to have this effect? The effect cannot be a simple immunosuppressive one because it continues so long after drug clearance. This continuing effect is a surprise, and its precise mechanism is a matter for conjecture and further study.

Lymphocytes in Murine Thyroditis. Dr. Yi Chi Kong discusses T lymphocyte proliferation and cytotoxicity in murine thyroiditis. Mice of appropriate H2 haplotypes immunized with mouse thyroglobulin in Freund's adjuvant develop thyroiditis with both cytotoxic T lymphocytes and helper T lymphocytes which proliferate when challenged in vitro with mouse thyroglobulin. She has nicely shown that Class I molecules restrict cytotoxicity for thyroid cell targets. T lymphocytes thus appear to play an important role in the pathogenesis of murine thyroiditis.

Moreover, in this murine system, Dr. Kong and her colleagues have been able to show suppressor T cell lymphocyte function which enhances or retards the experimental disease. Susceptibility to thyroiditis is certainly related to the major histocompatibility-complex. Conjecture about the nature of the molecular difference in

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the responses of different strains of mice is worthwhile. Is the difference a matter of antigen presentation, lack of response by helper T lymphocytes, differences in effect of suppressor T lymphocytes? What is the role of amplifiers in this system? What is the pathophysiologic role of cytotoxic T lymphocytes? Are these lymphocytes the most important cause of thyroid cell damage? And what is the relative importance of antibody-dependent cellular cytotoxicity?

Shared Antigens and Graves' Ophthalmopathy. Dr. Jack Wall next presents some of his fine studies using monoclonal antibodies as a probe to isolate various antigens in orbital tissues, with a view to finding antigens that bear on Graves' ophthalmopathy. He has succeeded in finding some antigens in orbital connective tissue and eye muscle which are shared with some thyroid antigens. One of the shared antigens appears to be a small component of thyroglobulin.

These studies are somewhat similar to others that have used monoclonal antibodies against fibroblasts that cross-react with the TSH receptor. Dr. Wall has speculated that the most likely mechanism for the association between Graves' ophthalmopathy and prior thyroid disease is that thyroid autoantibodies sometimes cross-react with shared antigens in eye muscles or orbital connective tissue.

However, Dr. Wall has not been able to show an association between any known thyroid autoantibodies and Graves' ophthalmopathy. Indeed, he published an earlier paper in *Lancet* (1982; 2:1353) demonstrating antibodies against orbital muscle in Graves' ophthalmopathy which could *not* be demonstrated in Graves' disease without ophthalmopathy, seemingly a point against his present contention. He has also recently shown that there is no significant amount of native thyroglobulin in eye muscle. Dr. Kendall-Taylor has also demonstrated antibodies to orbital issue in Graves' ophthalmopathy that are not demonstrable in Graves' disease without ophthalmopathy (Lancet 1984; 2:374).

The current findings of antigens shared by orbital tissues and thyroid tissues are intriguing, but in my view the relationship of these findings to Graves' ophthalmopathy should be scrutinized with some caution. Antigens shared by different tissues may well be common, but one should not hasten to impute pathophysiologic significance to such a finding. It is now clear that monoclonal antibodies can recognize the same epitope in many tissues, but this ability may not be relevant to the particular disease under investigation. However, the pursuit is worthy, and Dr. Wall may prove to be correct about this hypothesis.

The TSH Receptor and Its Antibodies. The recent work of Dr. Bernard Rees Smith has shown that the TSH receptor is composed of an A and a B subunit. The A subunit is a hydrophilic peptide located on the outside surface of the cell membrane; the B subunit must, therefore, contain the hydrophobic portion of the receptor, which transverses the cell membrane and is the likely site of interaction of adenylate cyclase. Dr. Rees Smith has demonstrated convincingly that TSH interacts directly with the receptor. His report here extends his evidence that TSH receptor antibodies also bind to the same site. Those who hold that such antibodies bind to other parts of the thyroid cell membrane, perturbing the TSH receptor from a distance, are having a more difficult time proving their point. The role of gangliosides in stimulation by the TSH receptor antibody of the TSH receptor, as reported by Kohn (Science 1978; 199:77) is not part of the model proposed by Rees Smith. Although this point will have to be resolved, his data have convincingly demonstrated the nature and structure of the TSH receptor, and I am personally convinced by his description.

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In Vitro Studies of Human Thyroid Autoantibody Synthesis

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In vitro studies of human lymphocyte function are usually carried out with lymphoid cells from the peripheral blood. These populations consist predominantly of virgin T and B cells or memory cells, and consequently they may not provide an accurate estimate of the active immune response occurring in secondary lymphoid organs like the spleen and lymph nodes. Furthermore, in autoimmune thyroid disease, thyroid lymphocytes appear to play a major role in autoantibody synthesis (1–3). Using previously established culture systems (4–7), we compared the ability of lymphocytes from the blood, thyroid, and lymph nodes of Graves' and Hashimoto patients to synthesize autoantibodies to thyroid antigens including thyroglobulin, thyroid microsomes, and the thyroid-stimulating hormone (TSH) receptor.

Methods and Materials

Thyroid tissue was obtained from seven Hashimoto patients (six women and one man; mean age, 50 yr) who were euthyroid on thyroxine and had microsomal and/or thyroglobulin antibody titers of >1:250 and >1:600, respectively, by hemagglutination analysis. Thyroid tissue was also obtained from 16 Graves' patients (13 women and 3 men: mean age, 32 yr) who had microsomal and/or thyroglobulin antibodies detectable by hemagglutination or enzyme-linked immunosorbent assay (ELISA) techniques; 9 patients were treated with carbimazole (5–45 mg/day), and 7 patients were controlled preoperatively with propranolol (160 mg/day). In addition to thyroid tissue, peripheral blood was obtained from 3 Hashimoto patients and lymph nodes draining the thyroid from 3 Hashimoto and 15 Graves' patients.

Blood lymphocytes were isolated by density gradient centrifugation (8), and thyroid and lymph node lymphocytes were extracted by teasing the tissue apart to release mononuclear cells; in most cases the resulting suspensions were separated from red blood cells and debris by using a density gradient, but in some cases it was only necessary to lyse the red cells in lymph node suspensions with buffered NH_4Cl. A different technique was used to extract lymphocytes from the thyroid specimen of two Graves' patients: the tissue was digested with dispase (9), and the resulting cell suspensions were washed and allowed to adhere to plastic overnight; the nonadherent cells were then removed, washed, and purified on a density gradient to give a suspension of thyroid lymphocytes. The viability of cell suspensions was assessed by trypan blue dye exclusion (10) and was >90% for blood lymphocytes and >80% for lymph node and thyroid lymphocytes. Lymphoid suspensions were cultured at 5×10^5 cells/ml in 1-ml aliquots in round-bottom tubes or at 7×10^6 cells in 1 ml in the inner chamber of Marbrook flasks (11) with 25 ml of medium in the outer reservoir; the culture medium was RPMI 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cultures were set up in duplicate or triplicate in medium alone or with po-

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keweed mitogen (PWM, 3 μl/ml, Gibco Grand Island). After 14 days at 37°C in an atmosphere of 5% CO_2 in air, cultures were harvested by centrifugation at 400g; the cell pellets were examined for the number of viable cells by using trypan blue and the supernatants were stored at −70°C until they were analyzed.

Culture supernatants were assayed for total immunoglobulin G (IgG) by a solid-phase radioimmunoassay (12) or an ELISA technique (13). Microsomal or thyroglobulin antibodies were determined by ELISA (6, 7). Optical density (OD) measurements obtained by ELISA were expressed as an index defined as

$$\frac{\text{OD of test sample (mean of two values)}}{\text{OD of reference serum (mean of two values)}}$$

The reference sample for microsomal antibody assays was a Hashimoto serum with a microsomal antibody titer of 1:204,000 (diluted 4,000 times) and the reference serum for thyroglobulin antibody assays had a thyroglobulin antibody titer of 1:5,120 and was diluted 400 times.

The distribution of microsomal or thyroglobulin antibodies among the IgG subclasses was determined by ELISA techniques (14, 15). The percentage contribution of each IgG subclass to microsomal or thyroglobulin antibody was taken as

$$100 \times \frac{\text{OD with individual subclass antibody}}{\text{total OD with all subclass antibodies}}$$

In the case of TSH-receptor antibody determinations, immunoglobulins in the culture supernatants were concentrated by precipitation with polyethylene glycol 4000 (final concentration 15%) (16). The precipitates were dissolved in 0.15 M NaCl, 10 mM Tris-HCl (pH 7.5) and assayed for inhibition of ^{125}I-labeled TSH binding to detergent-solubilized TSH receptors (16). Control values were obtained by using precipitates from culture medium only. In the presence of TSH receptors and "medium-only precipitates" about 30% of the labeled TSH was bound; binding in the absence of receptors (nonspecific binding) was about 3%. Results were expressed as inhibition of TSH binding, calculated as follows:

$$100 \times \left\{ 1 - \frac{\begin{array}{c}{}^{125}\text{I-labeled TSH specifically}\\ \text{bound in presence of}\\ \text{test culture concentrates}\end{array}}{\begin{array}{c}{}^{125}\text{I-labeled TSH specifically}\\ \text{bound in presence of}\\ \text{medium-only concentrates}\end{array}} \right\}$$

Results

The ability of lymphocytes from the blood, lymph nodes, and thyroid to synthesize IgG and microsomal or thyroglobulin antibody is illustrated for three Hashimoto patients in Fig. 1. Blood lymphocytes secreted small amounts of IgG when cultured in medium only and these levels were substantially increased when pokeweed mitogen was present during culture; further, in the case of patient JB, thyroid autoantibody synthesis was readily detectable only in pokeweed mitogen-stimulated cultures, and this is in agreement with previous observations (6, 17). Autoantibodies were not measurable in blood lymphocyte cultures from patient PG (despite synthesis of relatively large amounts of total IgG, and this was associated with a much lower serum titer of thyroglobin antibody in patient PG compared with patient BC (ELISA) index 0.27, compared with 2.27 in serum diluted 1/1,000). In contrast to blood lymphocytes, lymphocytes from the thyroid and lymph nodes of all three Hashimoto patients synthesized autoantibodies *spontaneously;* the effect of pokeweed mitogen on such cultures was variable, with a small rise, no change, or even decreased levels of thyroid autoantibody synthesis being observed (Fig. 1, D–F). Total IgG synthesis by lymph node lymphocytes was greater in the presence of pokeweed mitogen than in its absence (Fig. 1, A–C), and this is in accordance with results obtained from a much larger study (13). Thyroid lymphocytes from two patients produced increased amounts of total IgG when stimulated by pokeweed mitogen, and the third patient's lymphocytes showed a slight rise (Fig. 1, A–C); in a more extensive study we observed that thyroid lymphocytes may show no change or even decreased IgG synthesis when pokeweed mitogen is present during culture (13). Although IgG synthesis by thyroid lymphocytes in culture may vary considerably, thyroid autoantibody synthesis frequently occurs without the need for mitogenic activation.

Previously we showed that blood lymphocytes stimulated with PWM or Epstein-Barr virus synthesized thyroglobulin antibodies in culture which closely resembled thyroglobulin antibodies in serum in terms of their distribution among the IgG subclasses (14). As shown in Fig. 2, similar results were obtained with lymphocytes from the thyroid, lymph nodes, and blood of patient BC. Thyroglobulin antibodies secreted in culture were predominantly IgG_4 with smaller contributions from IgG_1, IgG_2, and IgG_3. It is of interest that the closest similarity with the proportions in

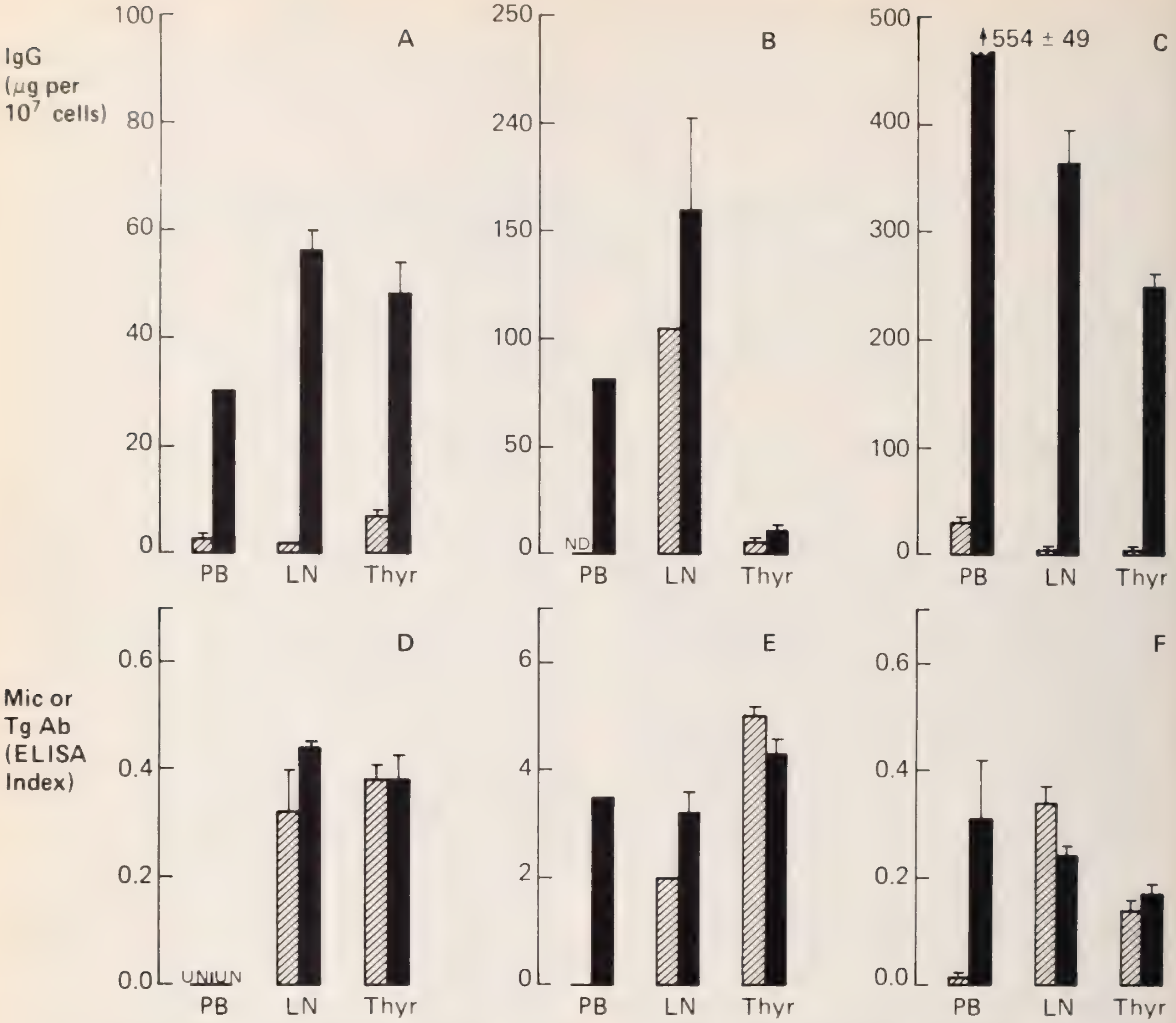

Fig. 1. Synthesis of IgG and microsomal (Mic) or thyroglobulin (Tg) antibody by lymphocytes from peripheral blood, lymph nodes, or thyroid of Hashimoto patient PG (**panels A and D**), patient BC (**panels B and E**), and patient JB (**panels C and F**). Lymphocytes from patients PG and BC were cultured in Marbrook flasks and from patient JB in tubes. Thyroglobulin antibody synthesis is shown for patients PG and BC and microsomal antibody for patient JB. Results are shown as mean of duplicates or mean ± standard error where triplicate cultures were available. Hatched bars, cultures without PWM; solid bars, cultures with PWM; ND, not determined; UN, undetectable.

serum was observed with *spontaneous* thyroglobulin antibody synthesis by lymph node or thyroid lymphocytes; the presence of pokeweed mitogen in lymph node or blood lymphocyte cultures was associated with a small but distinct shift toward IgG_1 (Fig. 2). These observations are in accordance with those of Mayumi et al (18) in studies of the IgG subclasses synthesized by peripheral blood lymphocytes from normal donors in the presence of pokeweed mitogen.

Figure 3 shows the IgG subclass distribution of microsomal and thyroglobulin antibodies present in serum and synthesized *spontaneously* in culture by thyroid lymphocytes from six Hashimoto and one Graves' patient; in all cases a close correlation existed between the antibodies present in vivo and those secreted in vitro. These results indicate that the antibodies in the circulation are an accurate reflection of those synthesized by lymphocytes in the thyroid and are not merely irrelevant antibodies "left over" after interaction with thyroid antigens.

Since both lymph node lymphocytes and thyroid lymphocytes are capable of spontaneous synthesis of thyroid autoantibodies, it was of interest to determine which of the two organs contributes to a greater degree to the circulating levels of thyroid autoantibodies. We examined this by comparing IgG and thyroid autoantibody synthesis by thyroid and lymph node lymphocyte suspensions isolated from Graves' patients treated preoperatively in different ways (19, 20). The results of our study, summarized in the Table, showed that *lymph node lymphocytes* from Graves' patients treated preoperatively with carbimazole for 1–2 years or 3–5 months or with propranolol had similar capacities to synthesize microsomal and/or thyroglobulin antibody in culture and to secrete IgG with or without pokeweed mitogen. In contrast, *thyroid lymphocytes* from Graves' patients treated for long periods of time with carbimazole had a significantly reduced capacity to synthesize IgG and thyroid autoantibodies compared with thyroid lymphocytes from patients treated preoperatively with propranolol (Table 1). Assessment of the numbers of viable cells after the 14-day culture interval demonstrated that this was associated with a decreased ability of thyroid lymphocytes (but not lymph node lymphocytes) from the carbimazole-treated patients to survive in culture (Table 1). Methimazole, the active metabolite of carbimazole, is immunosuppressive for IgG and thyroid autoantibody synthesis by cultures of peripheral blood lymphocytes (21, 22). Although the mechanism of action of carbimazole on the lymphoid system in vivo is not fully understood, it seems likely that the major effect is on lymphocytes localized within the thyroid gland. Since thionamide drug therapy is usually accompanied by a decrease in serum thyroid autoantibody levels, our investigations support the concept that the thyroid is the major site of human thyroid autoantibody synthesis.

Additional support for the role of thyroid lymphocytes in Graves' disease comes from studies of TSH-receptor antibody synthesis in lymphocyte cultures. We showed previously that TSH-receptor antibodies were synthesized by peripheral blood lymphocytes from Graves' patients in cultures containing pokeweed mitogen (4, 5). As illustrated in Fig. 4, we have now observed that thyroid lymphocytes from a Graves' patient treated preoperatively with propranolol (patient JS) synthesized TSH-receptor antibodies *spontaneously* (Fig. 4A), whereas her lymph node lymphocytes produced comparable levels only when stimulated with pokeweed mitogen (Fig. 4B). Similarly, lymph node lymphocytes from patient LW (who was treated preoperatively with carbimazole) produced detectable levels of TSH-receptor antibody only in the presence of pokeweed mitogen (Fig. 4B). These results for TSH-receptor antibody synthesis by thyroid and lymph node lymphocytes were essentially similar to those obtained for microsomal antibody production (Fig. 4, C and D); the ability of lymphocytes from the thyroid and lymph nodes of patient PS to secrete microsomal antibody but not TSH-receptor antibody was probably due to the high serum titers of microsomal antibody but low levels of TSH-receptor antibody in this individual (Fig. 4). Although part of the difference in ability of lymphocytes from thyroid and lymph node to synthesize microsomal and/or thyroglobulin antibody compared with TSH-receptor antibody may be attributable to differences in assay sensitivity, it is likely that the number of precursor B cells is lower in cells secreting TSH-receptor antibody.

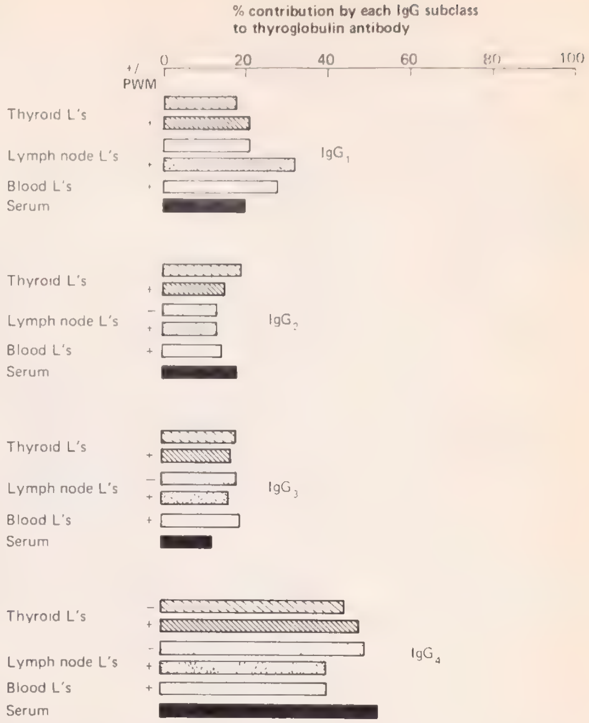

Fig. 2. Distribution among the IgG subclasses of thyroglobulin antibody present in serum and synthesized in culture with or without the addition of pokeweed mitogen (PWM) by lymphocytes from the blood, lymph node, and thyroid of Hashimoto patient BC.

Discussion

On the basis of the studies described here and those reported elsewhere, the pattern of synthesis of IgG and autoantibodies by lymphocytes from different lymphoid organs can be summarized as follows: lymphocytes in the blood produce little immunoglobulin in culture unless stimulated by pokeweed mitogen, and the cells that respond by synthesizing IgG and microsomal or thyroglobulin or TSH-receptor antibodies are probably memory B cells (4–6, 17, 23–25). Lymph node lymphocytes frequently produce microsomal and/

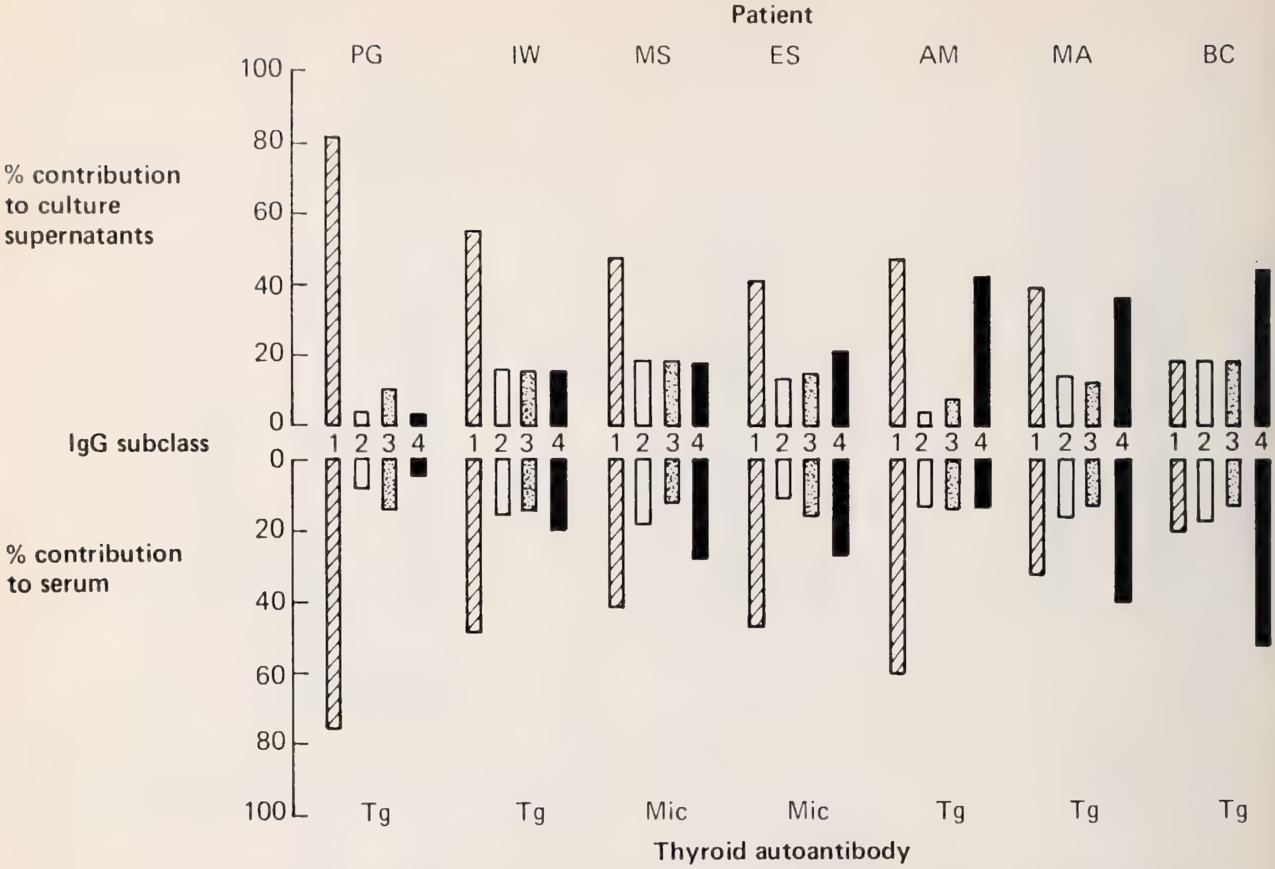

Fig. 3. Distribution among the IgG subclasses of microsomal (Mic) or thyroglobulin (Tg) antibody present in serum or synthesized in culture *spontaneously* by thyroid lymphocytes from six Hashimoto patients and a Graves' patient, AM.

or thyroglobulin antibodies spontaneously, and these levels may be increased when pokeweed mitogen is added to the cultures (19, 26). However, the magnitude of the increase is usually smaller than that observed for total IgG synthesis. Density gradient analysis of B cells showed that the precursors of microsomal or thyroglobulin antibody-secreting cells were different in blood and lymph nodes, and this is likely to be associated with the difference in the activation state of B cells in the two lymphoid organs (13). Thyroid lymphocytes from Graves' patients (treated preoperatively with propranolol) and Hashimoto patients may secrete thyroid autoantibodies (including TSH-receptor antibody) spontaneously in culture; the influence of pokeweed mitogen on such synthesis is variable but in a number of cases suppression was observed (1–3). As we suggested elsewhere (2, 27), this is likely to be due to activation of T suppressor cells and probably reflects an active immune response toward thyroid antigens. We have shown that the ability of thyroid lymphocytes to secrete thyroid autoantibodies spontaneously is due to the presence of activated B cell populations as well as activated T cells; in the absence of T cell suppression the lack of an increase in immunoglobulin or autoantibody synthesis in response to pokeweed mitogen appeared to be the result of a decreased proportion in the thyroid (compared with the blood) of T helper cells capable of being activated by PWM (27).

These studies of spontaneous autoantibody synthesis compared with synthesis induced by a T-dependent activator of B cells, pokeweed mitogen (28), demonstrate that the cell populations in blood, lymph node, and thyroid are at different stages of activation and contain functionally different proportions of regulatory T cells. However, the antibodies produced in culture by lymphocytes from these three lymphoid organs were essentially similar in terms of their distribution among the IgG subclasses, showing that the B cell *precursors* of thyroid autoantibody-secreting cells are the same.

In addition to providing information about the nature of the B cells capable of secreting thyroid autoantibodies, the IgG subclass distribution

TABLE

Comparison of Lymphoid Suspensions from Thyroid and Lymph Nodes of Graves' Patients Who Had Different Preoperative Treatments

	Group	Preoperative therapy	Thyroid lymphocytes	Lymph node lymphocytes
		A. Cultures without pokeweed mitogen		
Proportion of cultures positive for Mic/Tg Ab	I	Carbimazole, 1–2 years	0/6	3/6
	II	Carbimazole, 3–5 months	1/6	1/3
	III	Propranolol	2/4	2/4
IgG, μg per 10^7 cells	I	Carbimazole, 1–2 years	1.9 ± 1.1	12.9 ± 9.2
	II	Carbimazole, 3–5 months	0.9 ± 0.3	0.6 ± 0.1
	III	Propranolol	7.6 ± 4.8	3.3 ± 1.7
Percent recovery after culture	I	Carbimazole, 1–2 years	4.1 ± 3.1	16.8 ± 11.3
	II	Carbimazole, 3–5 months	6.3 ± 3.1	8.3 ± 5.2
	III	Propranolol	45.0 ± 17.8*	33.5 ± 23.6
		B. Cultures with pokeweed mitogen		
Proportion of cultures positive for Mic/Tg Ab	I	Carbimazole, 1–2 years	0/6	3/6
	II	Carbimazole, 3–5 months	1/3	1/3
	III	Propranolol	2/4	2/4
IgG, μg per 10^7 cells	I	Carbimazole, 1–2 years	5.7 ± 3.7	61.2 ± 20.3
	II	Carbimazole, 3–5 months	35.5 ± 34.8	59.1 ± 6.3
	III	Propranolol	34.7 ± 10.4**	52.6 ± 15.8
Percent recovery after culture	I	Carbimazole, 1–2 years	3.0 ± 0.4	26.2 ± 9.0
	II	Carbimazole, 3–5 months	9.4 ± 7.1	20.6 ± 5.1
	III	Propranolol	50.0 ± 15.8**	27.0 ± 8.0

Ability to secrete microsomal (Mic) or thyroglobulin (Tg) antibody (Ab) and total IgG and to survive culture (% recovery of viable cells) in lymphoid suspensions prepared from thyroid and lymph nodes of Graves' patients treated preoperatively with carbimazole for 1–2 years (six patients) or 3–5 months (three patients) or with propranolol (four patients). Values significantly differ from those for Group I patients: *$p < 0.05$; **$p < 0.01$.

of thyroid autoantibodies (particularly microsomal antibodies) may be relevant to the pathogenesis of the disease. We observed that the development of hypothyroidism in the postpartum period was associated with the level of microsomal antibodies of subclass IgG_1 but not IgG_4 (29).

Investigations of thyroid and lymph node lymphocytes from Graves' patients undergoing different forms of preoperative therapy demonstrated that thyroid lymphocytes (but not lymph node lymphocytes) from patients treated with carbimazole had a decreased capacity to survive in culture and to secrete IgG and microsomal or thyroglobulin antibody (19, 20). These results suggest that the major site of thyroid autoantibody synthesis is the thyroid, at least in Graves' patients, and additional support for this view is derived from our observation of spontaneous synthesis of TSH-receptor antibodies by lymphocytes from the thyroid gland.

In these studies it has been possible to determine the sites of active synthesis of thyroid autoantibodies, and we are characterizing the precursors of thyroid autoantibody production in terms of cell density, receptors for autoantigen, surface immunoglobulin class, and subclass of antibody secreted (13, 30, 31). It is also clear that the thyroid contains T cells capable of suppressing thyroid autoantibody synthesis, at least in culture. It should therefore be possible to utilize the in vitro system to explore the mechanisms that could be used to specifically decrease or even eliminate thyroid autoantibody-synthesizing cells in vivo. Immunospecific therapy of this kind might well provide a model for the treatment of other autoimmune diseases.

Summary

Lymphocytes from the blood, thyroid, and lymph nodes of patients with Graves' and Hashimoto's diseases have been assessed for their ability to synthesize autoantibodies to thyroglobulin, thyroid microsomes, and the TSH-receptor. These studies demonstrated that the precursors of thyroid autoantibody synthesizing cells are the same in all three lymphoid organs but their states of activation are different: the blood consists predominantly of memory cells which require activation with mitogen, whereas lymphocyte suspensions from both thyroid and lymph nodes are capable of secreting thyroid autoantibodies spontaneously. As serum levels of thyroid autoantibodies usually show a marked reduction during thionamide drug treatment, the effects of this

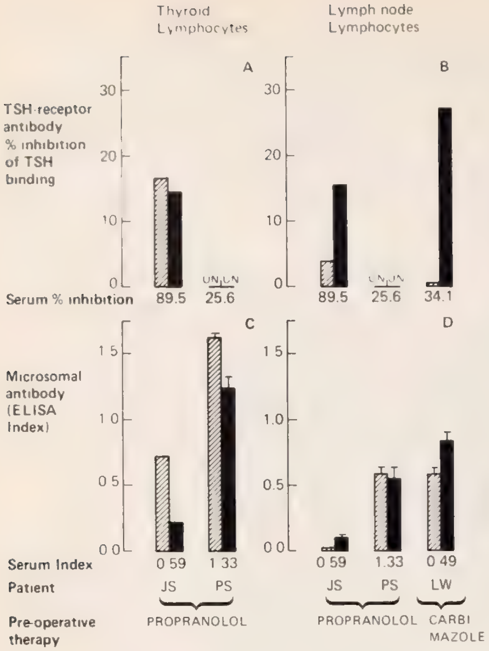

Fig. 4. Synthesis of TSH-receptor antibody and microsomal antibody in Marbrook cultures of thyroid lymphocytes (**panels A and C**) and lymph node lymphocytes (**panels B and D**) from Graves' patients JS and PS (treated preoperatively with propranolol) and patient LW (treated with carbimazole). Results for TSH-receptor antibody are shown as percent inhibition of TSH binding for immunoglobulins precipitated from pooled replicate Marbrook cultures; results for microsomal antibody are given as mean ELISA index for duplicate cultures or mean + standard error for triplicate cultures. Solid bars, cultures with PWM; hatched bars, cultures without PWM. Serum levels are given for TSH-receptor antibody (serum % inhibition) and microsomal antibody (serum index in test samples diluted 1/1,000). UN, values not significantly different from background levels for culture medium alone.

type of drug on thyroid autoantibody synthesis by the different lymphocyte populations were also investigated. A comparison of lymphoid suspensions from thyroid and lymph nodes of Graves' patients treated preoperatively with carbimazole or propranolol showed that the effect of the thionamide drug was predominantly on thyroid lymphocytes; this was consistent with previous evidence suggesting that thyroid lymphocytes are the major source of thyroid autoantibodies.

Acknowledgments

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In Vitro T Cell Proliferation and Cytotoxicity in Murine Autoimmune Thyroiditis

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Experimental autoimmune thyroiditis (EAT) in mice has served as a model for Hashimoto's thyroiditis (HT) because its induction is genetically based (1) and it exhibits characteristic autoantibodies to murine thyroglobulin (MTg) and mononuclear cell infiltrates in the thyroid. Induction of experimental autoimmune thyroiditis with the autoantigen, MTg, does not require emulsification with complete Freund's adjuvant (2); soluble, native MTg can be given intravenously either with bacterial lipopolysaccharide as adjuvant (3) or by itself in repeated doses (4). Analogy between Hashimoto's thyroiditis and experimental autoimmune thyroiditis is further illustrated by recent studies linking the HLA-DR region to HT (5) and pinpointing the immune response gene (Ir-Tg) for EAT to the H-2 I-A subregion (6). Moreover, genetic analysis of regulation of experimental autoimmune thyroiditis in H-2 recombinant strains has revealed that certain D-end genes reduce thyroid infiltration without influencing antibody levels (7). Such multiple controls more than likely exist in polygenic humans.

Numerous observations of a lack of correlation between antibody levels and the extent of thyroid damage in experimental autoimmune thyroiditis (8) have provided an impetus for the study of cell-mediated mechanisms in pathogenesis. Repeated injections of syngeneic MTg activate autoreactive T cells in vivo (4), and T cell lines can transfer disease to recipient mice (9). To characterize the T cell subsets involved and determine their relative role in thyroiditis, we recently developed two in vitro assays of T cell function applicable to experimental autoimmune thyroiditis. The first is a measure of the proliferative response of T cells to MTg stimulation (10, 11). Lymph node cells from immunized, EAT-susceptible mice respond to MTg activation in vitro, whereas those from immunized EAT-resistant mice do not, although antibody levels are high in both strains (11). We further found the proliferating cells to be Lyt-1-bearing T cells (12). Similar T cells proliferating in response to human thyroglobulin (HTg) have been observed in peripheral blood of Hashimoto's thyroiditis patients (13).

The second in vitro assay monitors the development of the cytotoxic capacity of a subset of T cells for syngeneic thyroid monolayers after in vitro culture (14); this subset is Lyt-2^+ but requires Lyt-1^+ cells for in vitro expansion and/or differentiation. Both the Lyt-1 and Lyt-2 phenotypes accumulate in the thyroids of mice that develop EAT at ratios different from those in the peripheral leukocytes (15). The cells that proliferate in vitro include clones that recognize unique and shared determinants of MTg and human thyroglobulin (HTg) (12) and are further characterized here. In addition, the capacity of concanavalin A (Con A) to expand the cytotoxic subpopulations of lymph node cells from immunized mice is determined.

Materials and Methods

Mice. Female CBA/J (H-2^k) mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. The animals were used at 8–10 weeks of age.

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Antigens. The procedure used for the preparation of murine and human thyroglobulins from thyroids obtained at necropsy has been described (7). Each thyroglobulin was purified from thyroid extract and its purity was verified by immunoelectrophoresis (12). The control antigen used in lymphocyte proliferation was purified protein derivative (Parke Davis and Co., Detroit, Mich).

Anti-I-A Monoclonal Antibodies. Anti-I-A antibodies were purified from ascites fluid by staphylococcal protein A separation in the laboratory of C. David. Monoclonal anti-I-A^k (Ia.15) was obtained from 17–227 hybridoma cells (U. Hammerling, Sloan-Kettering Institute, New York City) and anti-I-A^d was produced from hybridoma MKD6 (J. Kappler and P. Marrack, National Jewish Hospital and Research Center, Denver, Colo).

Immunization. Murine or human thyroglobulin was emulsified at a 1:1 ratio in complete Freund's adjuvant containing 3 mg/ml of *Mycobacterium tuberculosis* strain H37Ra (Difco Laboratories, Detroit). For the proliferation assay, each hind footpad was given 50 μl and each animal received 100 μg of MTg or 60 μg of HTg. For cytotoxicity studies, a total of 120 μg of MTg was given in 0.2 ml at four sites (both hind footpads and thighs).

In Vitro Proliferation Assay. The procedure used to study the proliferative response to MTg and HTg was essentially that described earlier (11, 12). Briefly, popliteal lymph node cells were obtained from mice 8 to 14 days after footpad immunization and cultured (4×10^5 cells per well) for 6 days at 37°C in an atmosphere of 5% CO_2 and 95% air. The medium was RPMI-1640 containing 25 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) (Gibco Laboratories, Grand Island, New York), supplemented with 1% normal syngeneic mouse serum, 2 mM glutamine, 50 μM 2-mercaptoethanol, penicillin (100 U/ml), and streptomycin (100 μg/ml). Varying concentrations of antigen (25 or 50 μg/ml) and irradiated (1500 R, ^{137}Cs-Gammacell 40, Atomic Energy of Canada, Ltd., Ottawa) spleen cells were added to the lymph node cell cultures. The cells were pulsed with 1.2 μCi of [^{3}H]thymidine (2 Ci/mmole, New England Nuclear, Boston, Mass) during the last 18 hr of culture and collected on glass fiber filters (Otto Hiller Co., Madison, Wis) to determine [^{3}H]thymidine incorporation. The results are expressed as the mean counts per minute (± standard deviation) of triplicate cultures. The effect of monoclonal antibodies to I-A on the proliferative response was determined by adding anti-I-A^k or anti-I-A^d (2 or 5 μg) to wells containing lymph node cells, irradiated spleen cells, and antigen at the time of culture.

Cytotoxicity Assay. The assay used to detect cells cytotoxic for thyroid targets was detailed previously (14). Popliteal and inguinal lymph node cells were harvested from mice immunized 12 days earlier. Cells (5×10^6/ml) were restimulated in vitro for 6 days with thyroid cells or with 10 or 25 μg/ml of MTg or 2 μg/ml of Con A (Difco Laboratories). Thyroid cell monolayers were derived from collagenase-disrupted thyroids as described previously (14). A cell suspension from the thyroids of 10–12 mice was used to seed the wells of a 96-well microplate (Corning Glass, Corning, NY) and was cultured for 5 days in the presence of 1 mM dibutyryl adenosine 3′,5′-cyclic monophosphate (cAMP) and 60 μU/ml thyroid-stimulating hormone (TSH), both from Sigma Chemical Co., St. Louis, Mo. Confluent monolayers were labeled with indium-111 (^{111}In) by incubation for 10 min with medium containing 50 μl of ^{111}In-Oxine (12 μCi/ml), which was prepared from ^{111}In-chloride at 2 mCi/ml (Medi-Physics, Inc., South Plainfield, NJ), following reported procedures (16).

After restimulation, lymph node cells were washed and added to wells containing the labeled thyroid target cells. Varying numbers (2×10^5 to 1×10^7) of effector lymph node cells were incubated with target cells in quadruplicate for 18 hr at 37°C. The supernatant fluids were then harvested and assayed for released ^{111}In in a gamma scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill). The percent specific release was calculated as 100 × (experimental release − spontaneous release) CPM divided by (maximum release − spontaneous release) CPM. The spontaneous release was determined by the radioactivity obtained when labeled target cells were incubated in medium alone, without added effector lymph node cells. The maximum release was determined by the radioactivity released when 1 N HCl was added to the target cells.

Characterization of Con A-activated cells with monoclonal antibodies to Thy-1.2, Lyt-1.1, or Lyt-2.1 and blocking of thyroid target cell recognition by rabbit anti-MTg serum and polyclonal antisera to H-2D and K antigens were carried out as described previously (14).

Results

Characteristics of the in Vitro Proliferative Response. The proliferative response to murine thyroglobulin, a self-antigen, is generally weaker

TABLE 1

*Effect of Irradiated Normal Spleen Cells on the Proliferative Response to Murine Thyroglobulin (MTg)**

No. of irradiated spleen cells per well	[^{3}H]Thymidine uptake, mean CPM ± SD (stimulation index)†			
	Medium	25 μg/ml MTg	50 μg/ml MTg	25 μg/ml PPD
None	2,000 ± 460	7,700 ± 1,100 (3.9)	12,800 ± 3,500 (6.4)	37,000 ± 5,600 (18.5)
4 × 10^5	2,000 ± 230	17,700 ± 5,700 (8.9)	20,600 ± 3,500 (10.3)	42,700 ± 2,100 (21.4)

* Lymph node cells from CBA/J mice immunized 14 days earlier with 100 μg of MTg in complete Freund's adjuvant were cultured (4 × 10^5/well) for 6 days with or without irradiated (1,500 R) normal spleen cells; [^{3}H]thymidine was added 18 hr before the cultures were harvested.

† Abbreviations: CPM, counts per minute; SD, standard deviation; PPD, purified protein derivative.

TABLE 2

Proliferative Responses to Shared and Unique Determinants on Murine Thyroglobulin (MTg) and Human Thyroglobulin (HTg) in Mice Susceptible to Experimental Autoimmune Thyroiditis

Immunizing antigen*	[^{3}H]Thymidine uptake, mean CPM ± SD (stimulation index)				
	Medium	25 μg/ml MTg	50 μg/ml MTg	25 μg/ml HTg	50 μg/ml HTg
MTg	10,900 ± 3,700	61,500 ± 4,400 (5.6)	60,700 ± 4,200 (5.6)	43,000 ± 12,200 (3.9)	59,300 ± 9,500 (5.4)
HTg	2,400 ± 340	4,700 ± 1,200 (2.0)	6,500 ± 1,340 (2.7)	80,900 ± 6,600 (33.7)	87,700 ± 4,700 (36.5)

* Lymph node cells from CBA/J mice immunized 8 days earlier with 100 μg of MTg or 60 μg of HTg in complete Freund's adjuvant were cultured for 5 days with irradiated normal spleen cells and varying concentrations of antigen.

TABLE 3

Effect of Anti-Ia Antibody on the Proliferative Response to Murine Thyroglobulin (MTg) and Human Thyroglobulin (HTg)

Immunizing antigen*	In vitro antigen	[^{3}H]Thymidine uptake, mean CPM ± SD (stimulation index)			
		None	2 μg anti-I-A^k	5 μg anti-I-A^k	5 μg anti-I-A^d
MTg	Medium	1,800 ± 450	1,900 ± 50	2,700 ± 330	2,700 ± 410
	25 μg/ml MTg	10,800 ± 3,100 (6.0)	1,980 ± 200 (1.0)	3,000 ± 160 (1.1)	10,800 ± 5,300 (4.0)
	50 μg/ml MTg	14,000 ± 2,600 (7.8)	2,290 ± 70 (1.2)	3,070 ± 100 (1.1)	13,300 ± 1,560 (4.9)
HTg	None	420 ± 50	ND†	ND†	ND†
	25 μg/ml HTg	27,500 ± 3,300 (19.5)	1,900 ± 760 (4.5)	600 ± 110 (1.4)	26,600 ± 5,100 (62)

* Lymph node cells from CBA/J (H-2^k) mice immunized 9 or 13 days earlier with 60 μg HTg or 100 μg MTg in complete Freund's adjuvant were cultured for 6 days with irradiated spleen cells and varying concentrations of antigen and monoclonal I-A antibody (dose/well).

† ND, not determined.

than the response to a foreign antigen, such as purified protein derivative (11). We recently examined the parameters involved in amplifying this in vitro assay (12). For example, the addition of irradiated spleen cells enhanced the response to MTg without affecting greatly the response to purified protein derivative (Table 1). We therefore routinely included irradiated speen cells in all proliferative assays in which lymph node cells were used. The presence of added speen cells occasionally increased the background counts in cultures without antigen, as seen in Table 2, but not so generally as to present a problem.

The proliferating lymph node cells were found to be Thy-1$^+$ and Lyt-1$^+$ (12). Table 2 shows that lymph node cells from MTg-immunized mice responded to in vitro stimulation with MTg as well as with HTg, indicating the presence

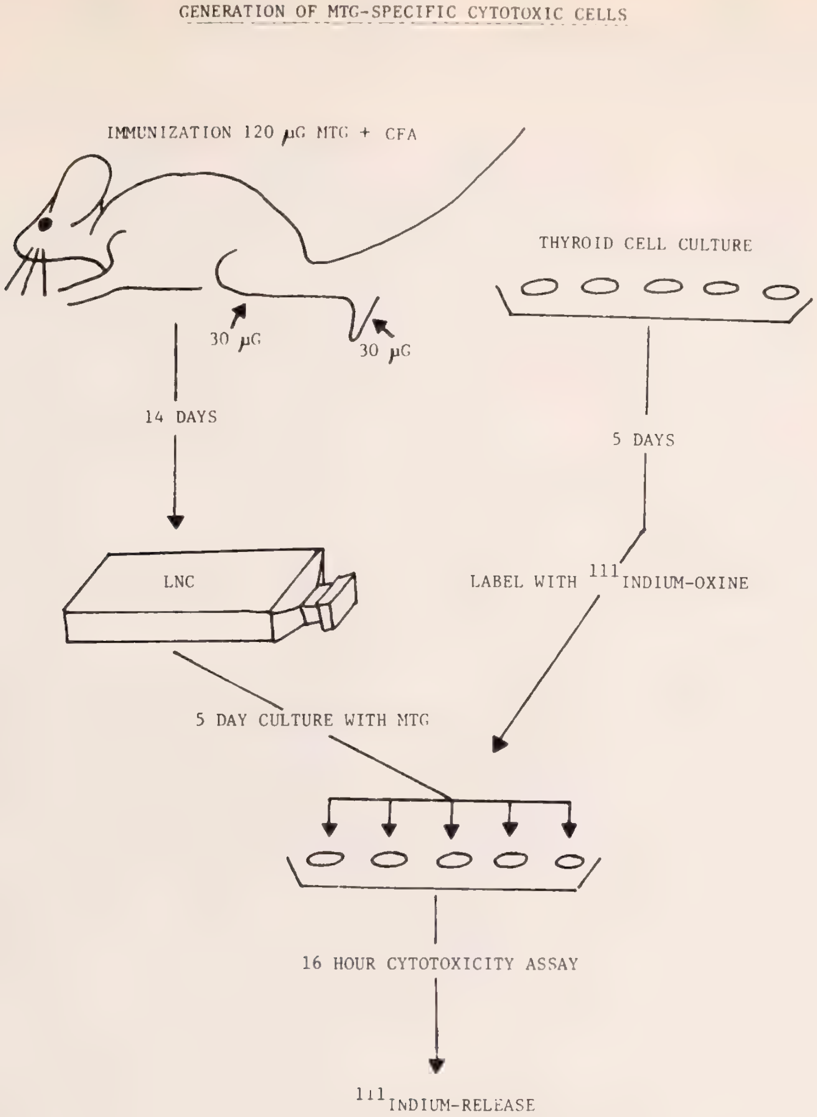
GENERATION OF MTG-SPECIFIC CYTOTOXIC CELLS
IMMUNIZATION 120 μG MTG + CFA
30 μG
30 μG
14 DAYS
THYROID CELL CULTURE
5 DAYS
LNC
LABEL WITH 111 INDIUM-OXINE
5 DAY CULTURE WITH MTG
16 HOUR CYTOTOXICITY ASSAY
111 INDIUM-RELEASE

Fig. Schematic diagram of cytotoxic T cell assay for lysis of thyroid target cells.

of T cells that recognized the shared determinants on the murine and human thyroglobulins. When the lymph node cells were from HTg-immunized mice, shared determinants on MTg were also recognized, but the majority of the cells proliferated in response to foreign epitopes of HTg to give high stimulation indices. The role of Ia antigens in proliferation was also investigated, since susceptibility to EAT has been localized to the I-A subregion of the MHC (6). Monoclonal antibodies to I-A^k antigens blocked the proliferative response to MTg; the control I-A^d antibodies had no effect (Table 3). The response of HTg-primed lymph node cells to HTg was likewise inhibited by the appropriate anti-I-A. When the proliferative responses to HTg were compared among independent haplotypes, the k haplotype appeared to be a high responder to both MTg and HTg (Krco CJ et al, unpublished observation).

T-cell Cytotoxicity for Thyroid Target Cells. We showed recently that lymph node cells from MTg-immunized mice became cytotoxic for thyroid monolayers (Fig.) upon further activation with MTg for 5–6 days (14). To determine whether thyroid cells themselves could provide the in vitro stimulation, primed lymph node cells were incubated with thyroid cells instead of MTg and were refractionated to serve as effector cells against freshly ^{111}In-labeled thyroid target cells. The thyroid cells were less efficient than MTg as an in vitro stimulator (Table 4), since higher numbers of effector cells were required to effect target cell destruction equivalent to that obtained after stimulation with MTg. The reason for the differ-

TABLE 4

*Cytotoxic Response of Primed Lymph Node Cells after in Vitro Stimulation with Murine Thyroglobulin (MTg) or Thyroid Cells**

Lymph node cell stimulation	No. of effector cells	Specific ^{111}In release (mean ± SD) from thyroid target cells (%)	
		Day 5	Day 6
10 µg/ml MTg	2×10^5	72 ± 8	59 ± 3
25 µg/ml MTg	2×10^5	61 ± 8	33 ± 2
Thyroid cells	2×10^5	20 ± 8	0 ± 3
Thyroid cells	1×10^6	36 ± 5	17 ± 5
Thyroid cells	2×10^6	48 ± 9	45 ± 12
Thyroid cells	1×10^7	59 ± 13	61 ± 15

* Lymph node cells were from mice immunized with 100 µg of MTg in complete Freund's adjuvant.

TABLE 5

Phenotype of Cytotoxic Cells after in Vitro Stimulation with Concanavalin A

Lymph node cell treatment	Specific ^{111}In release (mean ± SD) from thyroid target cells* (%)
Anti-Thy-1.2, rabbit antimouse immunoglobulin, and complement	35 ± 9
Anti-Lyt-1.1, rabbit antimouse immunoglobulin, and complement	68 ± 11
Anti-Lyt-2.1, rabbit antimouse immunoglobulin, and complement	18 ± 5
Rabbit antimouse immunoglobulin and complement	73 ± 14

* Measured on day 4 of culture.

TABLE 6

Specificity of Cytotoxic T Cells after in Vitro Stimulation with Concanavalin A

Target cell incubation	Dilution	Specific ^{111}In release (mean ± SD) from thyroid target cells* (%)
Rabbit anti-MTg absorbed with MTg-red blood cells	1:1	10 ± 7
	1:10	65 ± 8
	1:20	79 ± 15
Rabbit anti-MTg absorbed with spleen cells	1:1	5 ± 3
	1:10	23 ± 4
	1:20	45 ± 9

* Measured on day 4 of culture.

ence is unknown and may be related to the small amounts of MTg synthesized by cultured thyroid cells (14).

The cytotoxic cells generated in vitro by MTg activation were shown to be Thy-1$^+$, Lyt-2$^+$ (14). Furthermore, cytotoxicity was inhibited by the preincubation of thyroid target cells with MTg antibodies as well as antiserum to K or D (class I) antigens. To determine whether Con A activation generated cytotoxic cells with similar characteristics, 2 µg/ml of the mitogen was added to primed lymph node cells. As might be expected, Con A-generated cytotoxic cells were also Thy-1$^+$, Lyt-2$^+$ and were detectable at least 1 day earlier than when MTg was used (Table 5). The cytotoxic T cells recognized specific antigen on the thyroid target cells, since antibodies to MTg blocked specific ^{111}In release (Table 6). Only syngeneic target cells were destroyed by the Con A-activated cells, whereas thyroid targets from BALB/c (H-2^d) mice were not susceptible (data not shown). Furthermore, the Con A-activated cytotoxic cells showed class I antigen restriction similar to that of the MTg-activated cells (Table 7). Polyclonal antibodies to either the K or D region products reduced cytotoxicity by about 50% whereas their combined action abrogated cytotoxicity completely. Lymph node cells primed with saline in complete Freund's adjuvant and stimulated with Con A in vitro did not develop cytotoxic cells capable of killing thyroid monolayers.

Discussion

The findings described here illustrate the characteristics of the in vitro proliferative response of primed T cells to murine thyroglobulin activation in vitro. Addition of irradiated spleen cells enhances the response, which is relatively weak compared to the responses observed after in vivo sensitization and in vitro restimulation with the heterologous antigen, human thyroglobulin (Table 2). The proliferative responses of lymph node cells primed with either MTg or HTg are inhibited by I-A antibodies, which may interfere with antigen presentation. Thus, the lymph node cells primed with murine or human thyroglobulin contain T cell clones which recognize the shared epitopes on these thyroglobulins, as reported recently (12). In contrast to lymph node cells from EAT-susceptible mice, those from EAT-resistant mice primed with either murine or human thyroglobulin do not respond to in vitro activation with murine thyroglobulin (12). The absence of a detectable response of T cells from resistant mice

TABLE 7

Major Histocompatibility Complex Class I Antigen Restriction of Cytotoxic Response after in Vitro Stimulation with Concanavalin A

Immunizing antigen	Con A (μg/ml)	Target cell preincubation	Specific ^{111}In release (mean ± SD) from thyroid target cells* (%)
MTg-CFA†	2	Normal mouse serum	65 ± 11
		Mouse anti-H-2K^k	36 ± 11
		Mouse anti-H-2D^k	33 ± 9
		Mouse anti-H-2K^k + anti-H-2D^k	0 ± 10
Saline-CFA†	2		7 ± 11
	10		6 ± 5

* Measured on day 5 of culture.

† CFA, complete Freund's adjuvant.

suggests either that they lack T cells which recognize MTg-specific as well as shared epitopes, or that they have suppressor cells which inhibit T cell proliferation.

In the cytotoxicity system, thyroid cells, like murine thyroglobulin, can serve in activation in vitro to generate or expand T cells cytotoxic for thyroid target cells (Table 4). Murine thyroglobulin appears to be a more efficient stimulator, possibly because the amounts added to culture are much greater than the amounts synthesized by cultured thyroid cells, which are difficult to quantitate or enhance (14).

As with MTg-activated cells (14), Con A stimulation in vitro of MTg-primed lymph node cells results in expansion and/or differentiation of cytotoxic cells. These cytotoxic cells are similar to MTg-activated cells in that they are also Thy-1$^+$, Lyt-2$^+$ (Table 5) and display MHC class I antigen restriction (Table 7). Lymph node cells primed with saline, but not murine thyroglobulin, in complete Freund's adjuvant do not develop cytotoxic cells capable of destroying thyroid target cells. In addition to requiring murine thyroglobulin in sensitization, the Con A-activated cells show antigen specificity at the target cell level, since anti-MTg can block recognition (Table 6). Con A-activated lymph node cells from MTg-primed mice have recently been shown to transfer experimental autoimmune thyroiditis to normal recipient mice (17). We also compared the capacity of murine thyroglobulin and Con A to generate effector cells as demonstrated by in vivo transfer of experimental autoimmune thyroiditis (18). Greater numbers of Concanavalin A-activated cells than of MTg-activated cells were required to transfer the thyroiditis uniformly to recipient mice.

The in vitro proliferating cells and cytotoxic cells are Lyt-1$^+$ and Lyt-2$^+$, respectively. Both phenotypes accumulate in vivo in mice that develop experimental autoimmune thyroiditis (15). Some of these cells are likely to be antigen-specific, responding to further stimulation in the thyroid. It is not known whether the proliferation and cytotoxicity assays are in vitro correlates of in vivo pathogenesis. Accordingly, the role of cytotoxic T cells in initiating or mediating thyroid damage is unknown. It is likely, however, that both types of effector cells are involved. Further studies are in progress to determine their relative contribution.

Summary

Two in vitro assays for detecting T cell responses after in vivo sensitization with an autoantigen, murine thyroglobulin, are described. The proliferative response of Lyt-1$^+$ cells is enhanced by the addition of irradiated spleen cells. Lymph node cells from mice immunized with murine or human thyroglobulin proliferate in response to murine or human thyroglobulin, indicating the presence of T cell clones that recognize shared epitopes on both thyroglobulins. The proliferative responses of both sets of lymph node cells are inhibited by I-A antibodies. The cytotoxic response of lymph node cells primed with murine thyroglobulin is generated by activation with either murine thyroglobulin or thyroid cells. Concanavalin A can serve as a strong stimulator of cytotoxic cells which are Lyt-2$^+$ and display antigen specificity and major histocompatibility complex class I antigen restriction in target cell destruction. These assays may serve as in vitro correlates of in vivo pathogenic mechanisms.

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TSH Receptor Antibodies

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Hyperthyroidism in Graves' disease is due to autoantibodies to the thyrotropin (TSH) receptor (TSH receptor antibodies or TRAb) (1). Consequently, measurement of TRAb can be valuable in the diagnosis and management of thyroid disease. Furthermore, an understanding of the nature of the TSH receptor and its interaction with TRAb is essential to our understanding of the pathogenesis of Graves' disease. This short review considers some recent developments in these areas of investigation.

Measurement of TSH Receptor Antibodies

Originally, bioassay methods based on experimental animals were used to detect TSH receptor antibodies (2, 3), and more recently bioassay techniques using isolated thyroid cells in culture have been developed (4). The bioassay systems based on isolated thyroid cells do provide sensitive and specific methods for measuring the thyroid-stimulating activities of TRAb. However, they are relatively imprecise, expensive, and time-consuming and require a high degree of operator skill. Consequently, many laboratories have made considerable efforts to develop a "competitive binding" assay for TRAb.

There are several problems associated with this type of system. First, the TSH receptor represents a minute proportion of the total thyroid (or fat) membrane protein, and readily available preparations are heavily contaminated with other autoantigens (such as thyroglobulin), thyroid microsomal antigen, and cytoskeleton components (such as tubulin). Second, TRAb are a minute proportion of the patient's total serum IgG, and the serum IgG Graves' disease patients nearly always contains other autoantibodies such as those directed toward thyroid microsomes, cytoskeleton components, and thyroglobulin.

However, these problems have been successfully overcome by using the natural ligand for the TSH receptor: TSH itself. This material is available in pure form, can be labelled with iodine 125, and interacts specifically with the biologically active site on the TSH receptor. It provides the basis for a rapid, sensitive, precise, simple, and inexpensive method for measuring TRAb (5).

Briefly, detergent-solubilized TSH receptors are preincubated with test sera to allow any TRAb present to bind to the receptors. TSH labeled with iodine 125 is then added, and after a further period of incubation, receptor bound and free labeled TSH are separated by precipitation of bound hormone using polyethylene glycol. As TRAb and TSH compete with the biologically active site on the TSH receptor (see below), the presence of TRAb in the serum causes a reduction in receptor-bound labeled TSH. Recently, special sera calibrated against the MRC long-acting thyroid stimulator (LATS) standard B have become available, and TRAb measurements with the receptor assay can be expressed in terms of units per liter.

The receptor assay for TRAb is now used in many laboratories throughout the world. It can detect TRAb activity in virtually all patients with hyperthyroidism due to Graves' disease and is highly specific in terms of different disease groups (5).

Clinical Significance. The presence of TRAb in a patient with hyperthyroidism is indicative of Graves' disease, and the antibody is not detected in other forms of thyrotoxicosis (5). Recent studies have shown that the incidence of hyperthyroidism due to Graves' disease can show marked geographical variations relating to the past presence of endemic goiter (6). For example, in 1982

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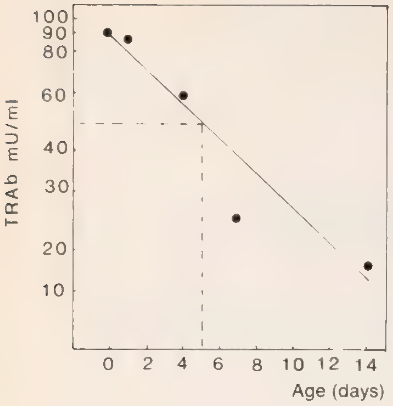


Fig. 1. Loss of TRAb activity from neonatal serum. Assays were carried out on 10μl aliquots of neonatal serum using method of Southgate et al (5) with standard serum calibrated against MRC LATS standard B. Baby studied was a patient of Dr. J. M. Wit, Utrecht, Netherlands, who kindly supplied serum samples.

in Preston, England, 35% of thyrotoxic patients were found to have Graves' disease, whereas in the same year over 90% of thyrotoxicosis in Southampton, England, was due to Graves' disease. Because treatment regimens for Graves' disease and for other forms of thyrotoxicosis differ, pretreatment measurement of TRAb can clearly be of value.

TRAb levels tend to fall during antithyroid drug treatment for Graves' disease, and if the antibody is absent after a course of drug treatment, remission of the disease process may have occurred and withdrawal of therapy can be considered. However, if TRAb levels remain high during antithyroid drug treatment, the disease is still active, and relapse is likely if the drug is withdrawn. In this circumstance destructive therapy can be considered.

Because detectable TRAb levels are indicative of active Graves' disease, TRAb measurements may also be useful in monitoring patients after partial thyroidectomy or radioiodine treatment.

About one half of patients with ophthalmic Graves' disease (eye signs of Graves' disease in the absence of a history of thyrotoxicosis) have detectable TRAb. TRAb measurements can be useful in distinguishing this form of eye disease from other forms such as orbital tumors.

TRAb are IgG-class antibodies and as such they can cross the placenta and cause neonatal hyperthyroidism (Fig. 1). Consequently, all pregnant women with a history of thyroid disease should be assessed for TRAb in order to assess the risks of neonatal thyrotoxicosis.

Properties of TSH Receptor Antibodies

Blocking. Comparison of the TSH receptor binding properties of TRAb with their ability to stimulate cyclic AMP release from cultured thyroid cells shows a generally good relationship between the 2 properties. However, occasional but marked discrepancies do occur. In our own laboratory, a few sera from patients treated with radioiodine have been shown to bind strongly to the TSH receptor but in such a way as to act as TSH antagonists. This blocking activity is present in the IgG fraction of sera and monovalent fragments of the IgGs are still potent TSH antagonists (Table).

There is increasing evidence that "blocking" antibodies acting as TSH antagonists can cause hypothyroidism, particularly after transplacental passage (10). It has been proposed that the blocking antibodies can cause hypothyroidism in adults with primary myxodema (11). In our own experience, however, this type of antibody is rare, and in a recent study of 40 patients with primary myxodema no blocking activity was detectable (using the techniques described in the Table). Similarly, TSH receptor binding activity was undetectable in this group of patients.

TSH Agonism and Antagonism. It has been proposed that sera of persons with Graves' disease contain a mixture of TSH receptor antibodies, some acting as TSH agonists and some as TSH antagonists (12). In order to investigate this possibility, we attempted to separate the two activities by isoelectric focusing. Five sera with thyroid-stimulating activity (Fig. 2) and two with TSH-antagonist activities (Fig. 3) were studied.

TABLE

TSH Antagonist Activity in IgG and IgG Fragments from Sera of Graves' Disease Patient Treated with Radioiodine

Sample	Cyclic AMP response (mean ± SE p moles per well; n = 5)
Normal IgG	0.32 ± 0.04
IgG + TSH	5.7 ± 0.1
Fab	0.32 ± 0.03
Fab + TSH	3.86 ± 0.56
(Fab)$_2$	0.7 ± 0.13
(Fab)$_2$ + TSH	2.7 ± 0.5
Patient IgG	0.26 ± 0.03
IgG + TSH	0.27 ± 0.05
Fab	0.14 ± 0.01
Fab + TSH	0.13 ± 0.02
(Fab)$_2$	0.32 ± 0.05
(Fab)$_2$ + TSH	0.3 ± 0.025

Bioassay based on the method of Kasagi et al (4); isolated porcine thyroid cells were employed in a hypotonic medium. Cyclic AMP released into medium in presence of IgG or IgG fragments (300 μg) with or without bovine TSH (10 μU; total incubation volume 200 μl) was determined by radioimmunoassay.

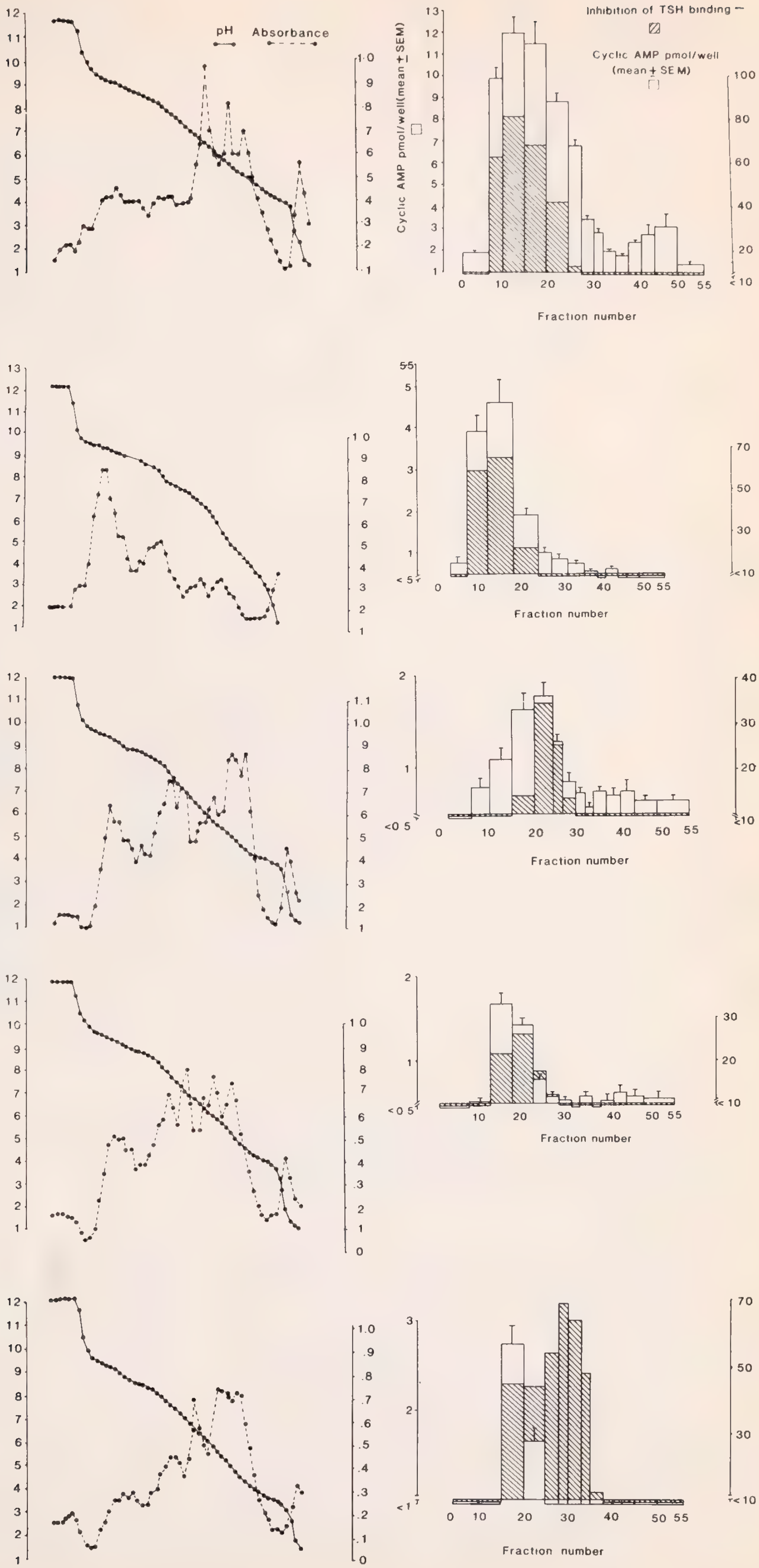


Fig. 2. TSH receptor binding and thyroid stimulating activity in isoelectric fractions of IgGs prepared from 5 patients with Graves' disease. IgG preparations (20mg; prepared by gel filtration on Sepharose G-200) were subjected to isoelectric focusing over pH range 3–10 using LKB 110ml column, 2% ampholine solution in sucrose gradient and voltage of 900 volts for 20h. Fractions were then dialysed against appropriate buffer and assayed by receptor assay (5) and bioassay (4, Table).

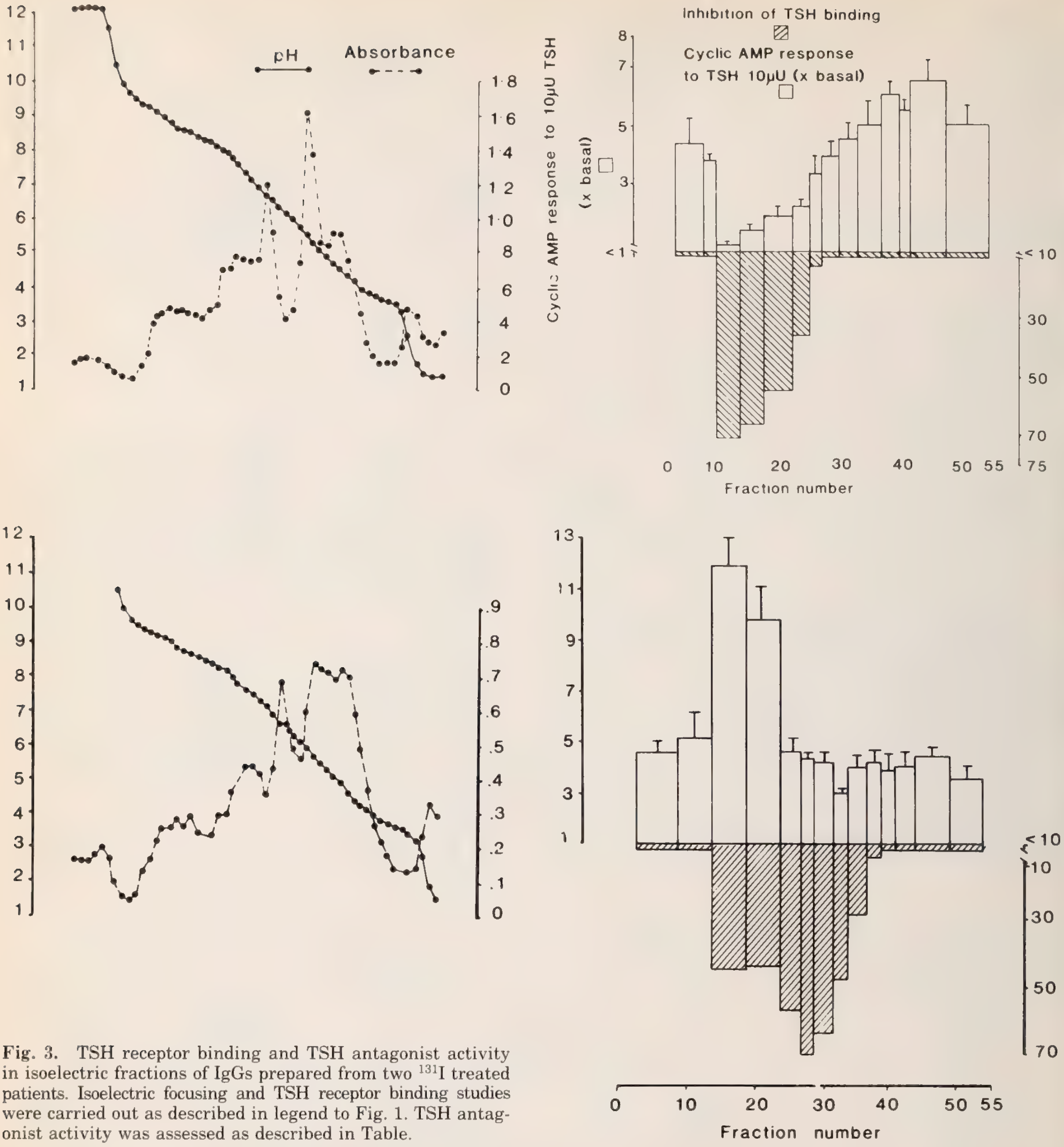
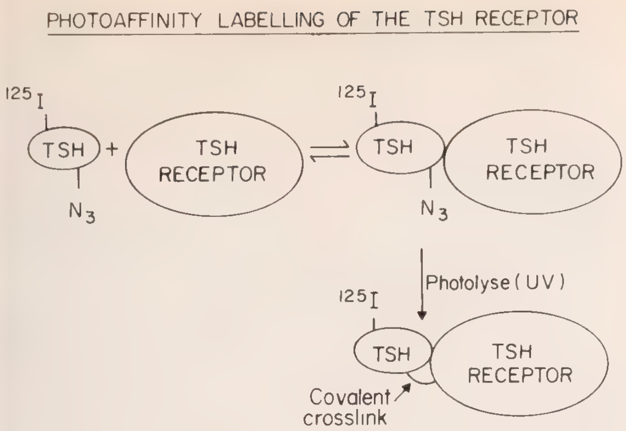


Fig. 3. TSH receptor binding and TSH antagonist activity in isoelectric fractions of IgGs prepared from two ^{131}I treated patients. Isoelectric focusing and TSH receptor binding studies were carried out as described in legend to Fig. 1. TSH antagonist activity was assessed as described in Table.

In all 5 Graves' sera IgG with thyroid-stimulating activity, this property was associated with a similar pH range, the peak of bioactivity being at pH 8.0–9.5. Similar results were obtained in early studies measuring LATS (13–15) or adenylate cyclase activity in thyroid slices (16).

In 4 of the 5, the isoelectric distribution of receptor binding and of thyroid stimulation was similar. In 2 of these, the proportions of receptor binding and thyroid stimulation in different fractions were fairly constant; in the remaining 2, the relative amounts of binding and stimulating activities varied. Presumably in the latter IgGs, isoelectric focusing had separated TSH receptor antibodies with different agonist activities. The occasional detection of low levels of receptor binding in the absence of thyroid stimulation may reflect the greater sensitivity of the receptor assay; alternatively, antibodies with weak binding but no TSH agonist activity may have been isolated.

The common isoelectric distribution of receptor-binding and thyroid-stimulating activities supports the hypothesis that they are properties



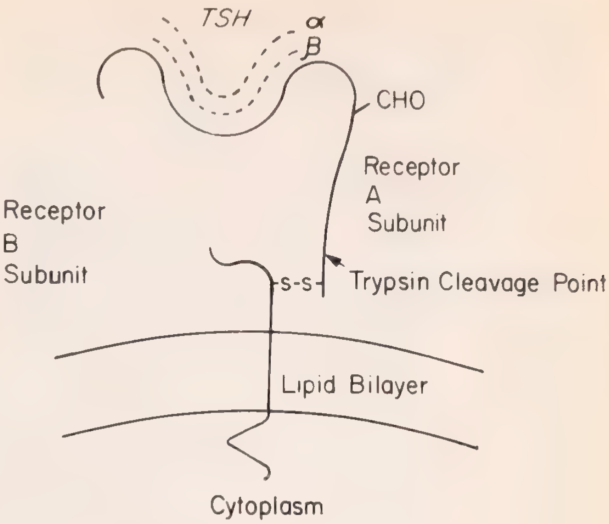


Fig. 4. **Left** Photoaffinity labeling of thyrotropin receptor.

Fig. 5. **Right** A structure for thyrotropin receptor.

of the same antibodies. The hydrophilic fragment of the TSH receptor (LAA) has a pI of 4.5 (13). The high pI of both receptor-binding and thyroid-stimulating activities is consistent with the observation that TSH receptor antibody binding and stimulating activity is absorbed by this subunit of the TSH receptor (13, 17).

The finding of isolated receptor-binding activity in the fifth Graves' IgG serum with thyroid-stimulating activity was more difficult to interpret. While the absence of thyroid-stimulating or blocking activities suggested that these fractions were not interacting with the TSH receptor, the receptor assay data suggest the opposite. At present these findings are not easily explained.

The 2 Graves' IgG preparations with TSH-antagonist activity had an identical isoelectric distribution of that property and receptor-binding activity. The ph range of both activities was similar to the range of the thyroid-stimulating activity in other Graves' IgG preparations. These findings are consistent with the recent observation that both thyroid-blocking and thyroid-stimulating antibodies bind to the same hydrophilic subunit of the TSH receptor (18).

In conclusion, it was not possible to separate either thyroid-blocking or thyroid-stimulating activities from TSH receptor binding activity by isoelectric focusing. The relatively restricted isoelectric distribution of TSH receptor antibodies contrasts with the wide isoelectric distribution of both thyroglobulin and microsomal antibodies (19).

Properties of the TSH Receptor

Several studies have shown that the TSH receptor has the following general properties:

It is an integral membrane glycoprotein readily soluble in nonionic detergents such as Triton X-100 or Lubrol 12A9 (20).

The receptor forms binding sites for TSH and TRAb on the outside surface of the thyroid cell membrane.

It forms a site for interaction with the regulatory subunits of adenylate cyclase, and this site is inside the lipid bilayer or at the cytoplasmic surface of the cell membrane (21).

The receptor is present on thyroid (20) or fat (21) tissues in extremely small amounts—about 1,000 sites per cell or about 10 ng/gm of wet tissue.

The low abundance of the TSH receptor means that special techniques are often required to study it. One of the most powerful of these techniques currently available is affinity labeling (23) (Fig. 4). This technique depends on coupling TSH to the photoactive crosslinking reagent N-hydroxy-succinimidyl-4-azidobenzoate (HSAB), labelling the HSAB-TSH with ^{125}I, and crosslinking the ^{125}I-HSAB-TSH to the TSH receptor by photoactivation of the azido group (24).

The method effectively labels the TSH receptor with ^{125}I by way of TSH, and the resulting stable complex can be analyzed by a variety of techniques. A particularly useful method is electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS-PAGE) followed by autoradiography. Such an analysis of affinity-purified porcine TSH receptors crosslinked to ^{125}I-HSAB-TSH has been described in detail (24–26) and indicates that under nonreducing conditions, crosslinked TSH-TSH receptor complexes have a relative molecular weight (M_r) of about 100,000; after subtraction of 28,000 for the M_r of TSH, this suggested that the receptor itself has an M_r of about 70,000. After mild reduction, however, the M_r of the crosslinked complex is about 73,000, giving an M_r for the reduced receptor of about 45,000. Consequently, mild reduction resulted in the loss from the receptor of a subunit of approximately 25,000 M_r which is not crosslinked to TSH. These results indicated that the TSH receptor contains 2 subunits linked by a disulfide bridge(s). One subunit (defined as the A subunit) has an M_r of about 45,000 and is crosslinked to TSH; the other (defined as the B subunit) has an M_r of about 25,000 and is not crosslinked to TSH.

When the crosslinked complexes are analyzed by sucrose density gradient centrifugation in the presence of the nonionic detergent Lubrol (0.1%), the crosslinked material sediments at a rate slightly slower than catalase (11S; M_r = 240,000), suggesting a structure considerably larger than the M_r of about 100,000 observed on SDS gels (25). However, the association of a large Lubrol micelle [M_r approximately 100,000 (27)] with the receptor during centrifugation would account for the apparent discrepancy.

Treatment of crosslinked complexes with 10mM dithiothreitol (DTT) prior to centrifugation caused a marked change in sedimentation characteristics, most of the radioactivity running between TSH and BSA (25). This suggested that after mild reduction, labeled TSH was associated with a much smaller structure, possibly due to the loss of the B subunit from the receptor together with the Lubrol micelle.

In order to investigate this possibility further, analyses were carried out using a calibrated column of Sephacryl S-300.

Nonreduced complexes were eluted as a symmetrical peak (K_{av} = 0.14) together with a small amount of free TSH (K_{av} = 0.50).

Complexes reduced in 10mM DTT prior to chromatography, however, showed a different elution profile with the appearance of a major peak (K_{av} = 0.32) eluting between the nonreduced complex and TSH. Analysis of this K_{av} = 0.32 peak on SDS-PAGE showed it to be the A subunit of the receptor crosslinked to intact TSH (M_r = 73,000) (25, 26).

To determine whether the A subunit of the receptor crosslinked to TSH (k_{av} = 0.32 peak) was associated with Lubrol micelles or not, Lubrol micelles were labeled with ^{3}H-oleic acid (^{3}H-oleic acid is spontaneously incorporated into Lubrol micelles) and run on the S-300 column. The micelles eluted from the column in a single symmetrical peak K_{av} = 0.27 (25, 26), indicating that the micelles had a considerably larger Stokes radius than the TSH-TSH receptor A subunit complex; consequently, the TSH-TSH receptor A subunit complex could not have been associated with a Lubrol micelle during chromatography. This suggested that the A subunit of the receptor was a hydrophilic peptide. As the intact receptor was associated with a Lubol micelle, these observations indicate that the micelle was associated with the B subunit of the receptor.

The region of the B subunit which binds to the Lubrol micelle will penetrate the cell membrane prior to detergent solubilization, and the data suggest a structure for the receptor (Fig. 5). If this model is correct, it should be possible to release the TSH receptor A subunit crosslinked to TSH from thyroid membrane preparations merely by the addition of reducing agent. This effect could be clearly demonstrated, confirming the proposed structure (25, 26).

Several comments concerning the structure are appropriate. There is a resemblance to the structure of the major histocompatability antigens (28). Preliminary studies with TSH receptor preparations from different species suggest that their structures are similar, with small differences in subunit M_rs between species (24, 29). Reduction of human thyroid homogenates release a water-soluble component of the TSH receptor (A subunit) which contains binding sites for both TSH and TSH receptor antibodies (18). The ability of the A and B subunits to dissociate readily after reduction of their interchain disulfide bridges indicates that interchain noncovalent interactions in the crosslinked complex are of relatively low affinity. The association of the B subunit with the cell membrane suggests that it is this subunit which is involved in interaction with the regulatory subunits of adenylate cyclase. These interactions may occur inside the lipid bilayer or at the cytoplasmic surface of the membrane (21).

Relation between Binding Sites for TSH and TRAb

The neutralization studies indicate that TSH receptor antibodies bind to the same subunit of the receptor as TSH (18). Furthermore, extensive immunoprecipitation studies have shown that partially purified, intact (containing both A and B subunits), solubilized TSH receptor cannot bind to TSH at the same time as TRAb (30).

This property of mutual exclusivity of binding suggests that the receptor antibodies do not interact with the B subunit. Furthermore, it indicates that the binding sites for the hormone and antibody on the receptor A subunit are closely related. Indeed, it seems likely that the antibodies bind to the same region of the receptor as TSH itself. This view is supported by the observed powerful TSH agonist action of many TSH receptor antibodies and their monovalent Fab fragments. To date it has not been possible to demonstrate any differences between the receptor binding characteristics of TSH receptor antibodies with TSH agonist or antagonist properties. Both types of antibody bind to the A subunit of the TSH receptor and neither type of antibody will bind to the receptor at the same time as TSH.

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Use of Mouse and Human Monoclonal Antibodies to Investigate the Immunologic Basis of Graves' Ophthalmopathy

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Monoclonal antibodies (MCABs) have proved to be powerful probes in studies of autoimmune disorders. They can be used to identify autoantigens, to isolate these antigens from crude tissue preparations, and to study the underlying basis of the autoimmune process. We have used a variety of mouse, and more recently human, MCABs against thyroid and orbital tissue antigens to study the immunologic basis of Graves' ophthalmopathy and the mechanism for its close association with autoimmune thyroid disorders.

Although it is generally accepted that Graves' ophthalmopathy is immunologically mediated (1–5), the nature of putative orbital tissue autoantigens and corresponding autoantibodies and the mechanism for its close association with Graves' hyperthyroidism and Hashimoto's thyroiditis are uncertain. Possible mechanisms for its association with the latter disorders include an effect of thyroid dysfunction on the immune system of predisposed patients, for which there is considerable evidence (6–8), precipitating a second autoimmune disorder; a metabolic effect of thyroid hormones on orbital tissue endothelial cells, adipocytes or fibroblasts, or on the extraocular muscle cells (9); a common genetic background leading to an enhanced association of these organ-specific autoimmune disorders; and autoimmune reactions against antigens shared by thyroid and orbital tissue (3, 5, 10). Although the recent findings of anti-eye-muscle antibodies in the serum of patients with Graves' ophthalmopathy (11–13) suggest that the eye disease is a separate autoimmune disorder associated with specific antigens, it is possible that the antibody activity reflects primary thyroid reactive antibodies cross-reacting with shared antigens in orbital tissue. That cross-reactive autoantibodies are a mechanism for the association of organ-specific autoimmune disorders was suggested by recent findings that MCABs prepared against purified human proteins often cross-reacted with other proteins and polypeptides (14–16). We investigated this possibility by using, as probes, mouse and human MCABs produced against human thyroglobulin (Tg), other thyroid antigens, and orbital tissue antigens, many of which were shown to cross-react with shared antigens in orbital or thyroid tissues, respectively. Of particular interest was the finding of an antigen site shared between human thyroglobulin and human orbital connective tissue membranes against which both antithyroglobulin MCABs and autoantibodies (from patients with autoimmune thyroid disorders) reacted. Results of this investigation are reported here. We also report results of studies in which we used a mouse MCAB to further purify the human eye muscle soluble antigen(S), against which 74% of patients with Graves' ophthalmopathy have circulating auto-

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antibodies (11), and attempted to isolate putative soluble Harderian gland autoantigens. Finally, we report preliminary results of our program in which human MCABs are used as probes to isolate eye muscle autoantigens.

Patients and Methods

Clinical Patients. Sera were obtained from the following groups.

Group 1: Forty-three patients with active Graves' ophthalmopathy, that is, in classes 3–6 in the American Thyroid Association classification (17, 18). Subjects included 6 men and 37 women aged 20–23 (mean age, 43), of whom 34 had associated hyperthyroidism at present (4 patients) or in the past (16 patients); 3 had associated Hashimoto's thyroiditis diagnosed from the characteristic goiter, significant titers (>1/256) of thyroid antibodies, and aspiration needle biopsy evidence of lymphocytic infiltration and Hürthle cells; and 6 had no associated thyroid disease (euthyroid Graves' disease).

Group 2: Eight patients with Graves' hyperthyroidism without eye disease, one male and seven females, aged 21–60 (mean age, 35).

Group 3: Eight patients with Hashimoto's thyroiditis without eye disease, one male and seven females, aged 22–67 (mean age, 49).

The sera from these groups were studied for antibodies against thyroglobulin, Harderian gland, and orbital connective tissue antigens. Sera from 30 normal subjects, 10 male and 20 female, aged 21–55 (mean age, 35, were also studied as controls.

Antigen Preparation. Normal human orbital tissue (eye muscle, orbital connective tissue, and lacrimal gland), skin, and liver were obtained at autopsy less than 4 h after death. Normal human thyroid tissue was obtained at operation from patients undergoing surgery for removal of single (nonmalignant) nodules. Tissues were rinsed, cleaned of connective tissue, finely minced with scissors, and homogenized in a mechanical blender. For the preparation of soluble and membrane fractions homogenates were first centrifuged at 400 × *g* for 15 min to separate off whole cells, debris, and nuclei and the supernatant was centrifuged at 100,000 × *g* for 30 min. The final supernatant ("cytosol") was retained and the pellet ("membranes") washed, twice, in phosphate-buffered saline. All cytosol and membrane fractions prepared were divided into aliquots and stored at −70°C until they were used.

Isolation of Eye Muscle Cells. Whole, viable, eye muscle cells were prepared from fresh normal pig eye muscle tissue by digestion of finely teased tissue with collagenase and trypsin. Free cells were obtained from the digestion mixture by centrifugation at low speeds. The pellet was discarded and the supernatant carefully layered onto a four-stage percoll gradient and centrifuged at 3,000 rpm for 30 min at 20°C. Several discrete bands were obtained and allowed the isolation of free eye muscle cells (band II) from connective tissue cells (band III), mononuclear cells (band IV), and debris (band I).

Sephadex G-200 Chromatography. Human eye muscle-derived soluble antigen, S, was partially purified from crude cytosol by chromatography on a 2 × 30 cm Sephadex G-200 (superfine) column equilibrated and eluted with phosphate-buffered saline (pH 7.4). Aliquots of 3.7 ml were collected. Antigen peaks were identified by reaction with MCAB 50-24. Positive aliquots were pooled, freeze-dried, and stored at −75°C until use. Further purification of S will be described in the section on results.

Affinity Chromatography. An affinity column for purification of S was prepared by coupling 10 mg of MCAB 50-24-immunoglobulin to 2 ml (0.6 g) of CNBr-activated Sepharose. Partially purified, freeze-dried antigen was then incubated in a beaker with the immunoglobulin Sepharose 4B gel overnight at 4°C, and the gel was poured into a 0.9 × 15 cm column. After the gel was washed with 0.15 M NaCl and 0.6 M NaCl in phosphate buffer, pH 7.4, the specific immunoglobulin G (IgG) was eluted with 0.2 M glycine and 0.6 M NaCl, pH 2.4.

Thyroglobulin was prepared from the soluble (cytosol) fraction of normal human thyroid tissue by the method of Van Herle et al (19), in which soluble proteins are precipitated by ammonium sulfate and the washed pellet chromatographed on Sephadex G-100. The first peak, containing thyroglobulin, was collected, concentrated on Sephadex G-75, and rechromatographed on Sepharose CNBr. The second peak (thyroglobulin) was collected and stored at −70°C until use.

Monoclonal Antibody Production. Mouse monoclonal antibodies against human thyroglobulin, human thyroid membrane antigens, and human orbital tissue antigens were produced as described previously (11). Briefly, adult BALB/c mice were immunized weekly for up to 12 weeks with thyroglobulin, thyroid membranes, or cytosol and membrane fractions of orbital connective tissue and eye muscle; they were given a final booster three days before fusion, which was carried out when serum antibodies reached peak levels. Spleen cells (10^8) were fused with SP-20 mouse myeloma cells (10^7) in 30% polyethylene glycol by the classical method of Köhler and Milstein (20). Seven (A1, A3, A7, A18, A25, A53, and A71), whose characteristics have been described in detail (11), were used for the present studies. In addition, we tested a further ten mouse MCAB-Tg's supplied by J.R.; their nature, binding affinities, and immunological characteristics were recently described (21). All 17 MCABs reacted strongly in enzyme-linked immunosorbent assays (ELISAs), immunofluorescence tests, and radioimmunoassays with all forms of human thyroglobulin tested. Human MCABs were produced by fusion of peripheral blood lymphocytes from patients with ophthalmopathy, with or without associated autoimmune thyroid disorders, with a human myeloma cell line (GM 4572B, National Institute for General Medical Sciences, Camden, New Jersey). The procedure used was similar to that described above; the only modification was that, in order to monspecifically enhance antibody-producing B cells, the peripheral blood lymphocytes were infected with Epstein-Barr virus (EBV) before fusion. Supernatants from more than 50 EBV-transformed cell lines were tested, three to four weeks after infection, for antibodies against thyroid and orbital tissue antigens. These cell lines have not yet been cloned. Recently, hybridizations have been carried out between 22 of these EBV-transformed cell lines and the human myeloma cells at a ratio of one lymphocyte to one GM 4572B cell. Hybridomas were screened with an ELISA for orbital and thyroid antibody production and then cloned, by limiting dilution, prior to expansion and further characterization. Thyroid and orbital antibody-producing cell lines and hybridomas and clones were studied for orbital tissue-thyroid cross-reactivity.

Monoclonal Antibody Characterization. Monoclonal antibodies, produced in mouse/mouse and human/human hybridization systems, were characterized with respect to affinity with antigen; immunoreactivity with antigen in crude immunizing preparations and with partially purified antigens (as identified) in ELISA and immunofluorescence tests; Ig class and IgG subclass as determined by Ouchterlony immunoprecipitation with antimouse or antihuman Ig-class and IgG-subclass antisera, respectively, and by an ELISA with the same antibodies linked to appropriate enzymes; ability to fix complement, determined in a classical sheep red blood cell, anti-sheep red blood cell system with guinea pig serum as source of complement, and in immunofluorescence tests with FITC-labeled anti-C3 β conjugate; and tissue and species specificity determined with an ELISA. Immunoglobulin was prepared from culture supernatant or ascites fluid by DEAE$^+$-cellulose chromatography. Immunoglobulin G is present in the first peak, and immunoglobulin M and other proteins are eluted in the second peak. Immunoglobulin-containing aliquots were pooled, freeze-dried, and reconstituted just before use.

Enzyme-Linked Immunosorbent Assay. ELISAs were used to screen culture supernatants from hybridomas and cell lines for thyroid and orbital tissue antibodies; to characterize MCABs with respect to Ig class, IgG subclass, tissue and species specificity, and reactivity with specific antigens prepared from soluble, membrane, and solubilized membrane fractions; and to test reactivity of sera with thyroid and orbital tissue antigens. Standard tests were used. Briefly, antigen was adsorbed, at 4°C overnight, to the surface of plastic microtiter plates previously treated with glutaraldehyde (0.1%) in the case of membranes. After washing, uncoupled antigen reactive sites were blocked by incubation with 3% bovine serum albumin. Antigen was then incubated with MCAB or serum for 2 hr at 37°C. After washing, second antibody (anti-mouse or anti-human IgG conjugated to alkaline phosphatase, Boehringer Mannheim Biochemicals, No. 60527, West Germany) was added and the plates incubated for a further 2 hr at room temperature. Finally, substrate (dinitrophenol) was added and the reaction arrested after 30 min for soluble antigens, and after 20 min for membrane antigens. Antigen concentration was generally 50 μg/ml. In some experiments dose-response studies were performed with antigen concentrations ranging from 0.1 to 100 μg/ml. The MCAB and serum dilution was 1/100, which was found, in preliminary experiments, to be optimal for reaction with thyroid and orbital tissue antigens. Tests were set up in triplicate and read at 420 nm in a micro-ELISA reader, results being expressed as optical density. A positive test was determined as an optical density of more than twice background (tests with complete medium for MCAB and phosphate-buffered saline for sera). This was 0.30 for MCAB and 0.25 for serum antibodies.

Other Methods. Protein concentrations were determined by the Biorad method with human gamma globulin as standard. Thyroglobulin and microsomal antibodies were also measured by using standard commercial hemagglutination test kits (thymune "M," thymune "T," Burroughs Wellcome Ltd., Beckenham, UK).

Statistical Analysis. Serum antibody levels against orbital connective tissue (OCT) membrane and Harderian gland antigens in patients

TABLE

Characteristics of Mouse Monoclonal Antibodies against Human Thyroid and Orbital Antigens, Pig Eye Muscle Cells, and Guinea Pig Harderian Gland Antigens

						Specificity		
Immunizing preparation	No. of positive clones	Primary antigen*	Ig class	IgG subclass†	Complement fixation	Species	Tissue	Cross-reactive
Thyroid								
Cytosol	20	Tg(19)-colloid	IgG	IgG_1	Yes	4/8	3	16
		antigen II(I)	IgG	IgG_1	NT	NT	1	0
Extract	20	Tg(18)	IgG	IgG_1	Yes	5/10	0	18
		Mem(2)	IgG	IgG_1	NT	NT	2	0
Eye muscle								
Cytosol	8	Cyt	IgG	IgG_1	2/8	7/8	3	5
Membranes	8	Mem	IgG	IgG_1	1/2	0/2	2	7
Extract	10	Mem	IgG	IgG_1	NT	NT	10	0
Eye muscle								
Solubilized membranes	8	Mem	NT	NT	NT	NT	NT	NT
Pig eye muscle cells	2	Cell Wall	NT	NT	NT	NT	NT	NT
Orbital connective tissue								
Cytosol	2	Cyt	NT	NT	NT	NT	2	0
Membranes	3	Mem	NT	NT	NT	NT	2	1
Harderian gland								
Cytosol	2	Cyt	NT	NT	NT	NT	2	0
Membranes	1	Mem	NT	NT	NT	NT	1	0
Lacrimal gland								
Cytosol	2	Cyt	NT	NT	NT	NT	2	0
Membranes	2	Mem	NT	NT	NT	NT	2	0

* Mem, membranes; Cyt, cytosol.

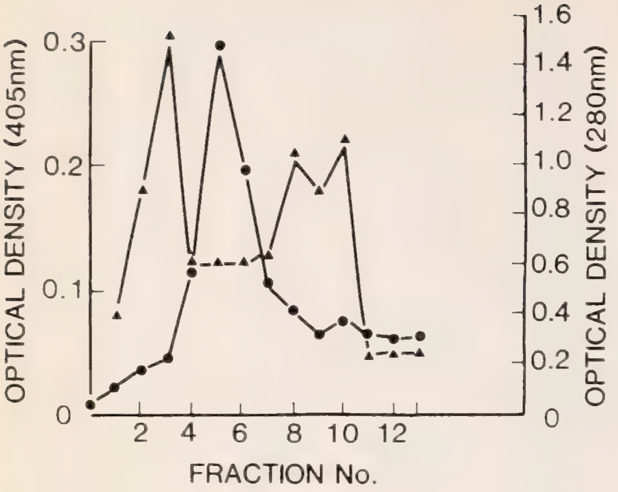
† NT, not tested.

and normal subjects were compared for statistically significant difference by using Student *t* tests. Correlation between anti-thyroglobulin and anti-OCT membrane antibody levels was determined as a correlation coefficient (r), significance being expressed as $p < 0.05$.

Results

We have produced over 200 mouse and human MCABs against human thyroid and orbital antigens. The characteristics and antigen reactivities of mouse MCABs are summarized in the Table. As described below, most of these were not tissue-specific. Our studies have focused on certain of these MCABs, namely MCAB 50-24, an antibody against eye muscle soluble antigen, S; three MCABs against Harderian gland antigens; and 17 MCABs against thyroglobulin, most of which were shown to be cross-reactive with an antigen in human orbital connective tissue membranes.

Purification of Human Eye Muscle Soluble Antigens (S). Further chromatography of Sephadex G-200-isolated human eye muscle soluble antigen, S, on the anionic exchanger DEAE$^+$-Sephacel resulted in two protein peaks; however, the antigen reactive with MCAB 50-24, the soluble eye muscle-derived autoantigen S, was confined to the smaller of these peaks. Transfer of the antigen fraction to sodium dodecyl sulfate (SDS) gels showed that the antigen reactivity did not correspond to the major protein peak found with crude cytosol, but still contained two, less prominent, bands. The pooled fraction was then further chromatographed on the cationic exchanger carboxymethylcellulose (CM52). The column, equilibrated with 50 mM acetate, was eluted with a linear gradient of NaCl and 4.0-ml fractions were collected. The peak of antigen reactivity was found in fractions 12–19, which contained only traces of protein as shown by absorbance at 280 nm and not enough to give a positive band with Coomassie Brillant Blue on SDS gel. When attempts were made to further purify S by affinity chromatography, using MCAB 50-24-Ig coupled to CNBr-activated Sepharose 4B by elution at pH 2.2, the peak of reactivity (Fig. 1), determined by ELISA, did not contain enough pro-



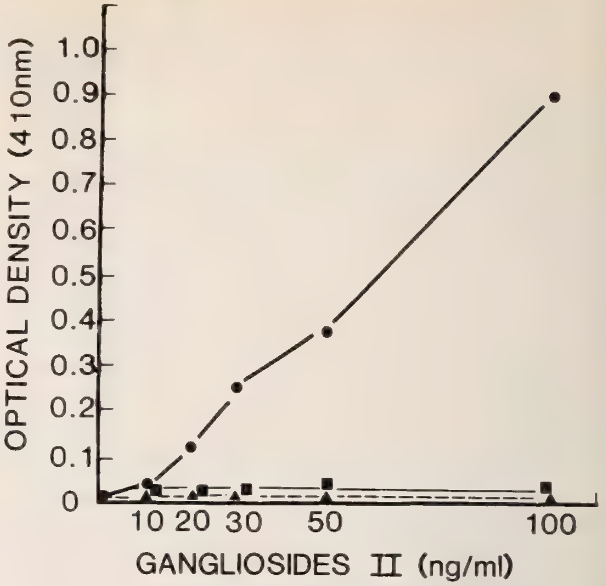


Fig. 1. Left Affinity chromatography of human eye muscle soluble antigen, S. Affinity column was prepared by coupling 10 mg of MCAB 50-24 thyroglobulin to 2 ml of CNBr-activated Sepharose 4B. Nonpurified human eye muscle cytosol was applied to column (0.9 × 15 cm) and specific IgG eluted with 0.2 M glycine-HCl, 0.6 M NaCl, pH 2.4 Reactivity of each fraction with 50-24 was determined with an ELISA (●). Results are expressed as optical density at 405 nm. Protein concentration (▲) is expressed as optical density at 280 nm.

Fig. 2. Right Reactivity of MCAB 50-24 with crude ganglioside mixture assessed in an ELISA (●). MCABs 79-1 (against human eye muscle cytosol) (■) and 64-1 (Harderian gland cytosol) (▲) were tested as controls.

tein to give a positive reaction with electrophoretic protein dye. In contrast to mouse polyclonal antibodies against S (tested as controls), patient autoantibodies and MCAB 50-24 did not convincingly precipitate with Sephadex G-200-purified S as determined by Ouchterlony double immunodiffusion (results not shown).

On the basis of this evidence it seems likely that the soluble antigen S, recognized by both MCAB 50-24 and serum autoantibodies from patients with Graves' opthalmopathy, is not a major protein. Indeed, 50-24 reacted in an ELISA with a crude ganglioside mixture (Sigma ganglioside II) derived from neural tissue over the range 0–100 μg/ml (Fig. 2). This suggest that the antigen is not eye-muscle-specific.

Harderian Gland Antigens. Guinea pig Harderian gland MCABs were used to identify, and partially purify, antigens in crude preparations of Harderian gland cytosol. These antigens were then used in an ELISA to test for corresponding autoantibodies in the serum of patients with Graves' ophthalmopathy. When Harderian gland cytosol was chromatographed on Sephadex G-200 and aliquots tested in an ELISA for reactivity with antibody 63-19, two antigen peaks (peaks II and IV, Fig. 3) were identified. These, along with control pools I and III, were concentrated and used as antigens in an ELISA to test sera from patients with Graves' ophthalmopathy for corresponding autoantibodies. As can be seen in Fig. 4, although there were no significant differences between patients with Graves' ophthalmopathy and normal subjects for any of the four fractions (*t* tests, not significant), 4 patients out of 20 (20%) had values above the upper limit of normal (mean + 2 standard deviations for normal subjects) in tests with pool IV.

Antigens Shared by Thyroid and Orbital Tissue. Sixty-six mouse MCABs, 44 against human thyroid antigens and 22 against human orbital tissue antigens, and 113 human MCABs were tested for thyroid and orbital tissue cross-reactivity. Thirty-three (50%) of the mouse MCABs and 16 (13%) of the human MCABs reacted with one or more antigen sites shared between thyroid gland and orbital (eye muscle, connective, lacrimal gland) tissue. Seventeen mouse MCABs against thyroglobulin (seven A series, ten J series) were studied in more detail. These were tested in an ELISA for reactivity against cytosol and membrane fractions of various human tissues, including orbital connective tissue, lacrimal gland, eye muscle, thyroid, and liver. Surprisingly, a large proportion of these antibodies cross-reacted with an antigen in orbital connective tissue mem-

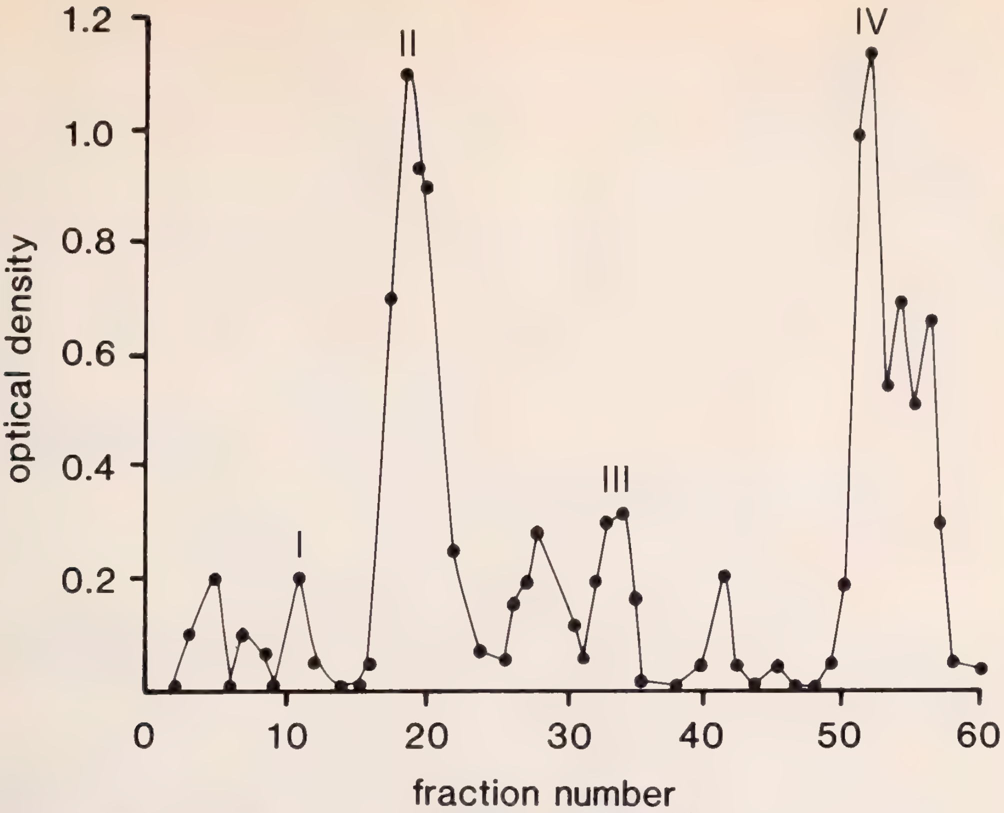


Fig. 3. Sephadex G200 chromatography of guinea pig Harderian gland cytosol fraction. Crude cytosol was applied to column (0.9 × 15 cm) of Sephacel S200 and eluted with linear gradient of 0–10 M NaCl at flow rate of 2.8 ml/hr. Aliquots (1 ml) were collected and tested for reactivity with MCAB 63-19 in an ELISA. Results are expressed as optical density at 410 nm. Two positive peaks (II and IV) and two negative pools (I and III) were collected and used as antigens in tests with sera from patients with Graves' ophthalmopathy and normal subjects.

branes. Although reaction of anti-thyroglobulin MCABs with OCT membrane prepared from different normal subjects was variable, all but one of the 17 MCABs reacted with at least one of the 14 preparations of OCT membrane tested (results not shown). Reactivity of one of these MCABs, A3, with OCT membrane 1 was completely absorbed by incubation, at 37°C overnight, with OCT membrane 1 and thyroglobulin but not OCT membrane 2 (Fig. 5). Although none of these antithyroglobulin MCABs reacted with connective tissue membranes prepared from bowel or skin, all, except A3, reacted strongly with all preparations of liver membranes tested (data not shown). The thyroglobulin shared reactive site was not native thyroglobulin since reactivity was not found in the cytosol fraction and it could not be solubilized. There were no significant statistical correlations between the antithyroglobulin and anti-OCT membrane activities (expressed as optical density) for the 17 MCABs when either anti-OCT membrane 1 (r = 0.37, p not significant), or anti-OCT membrane (12) (r = 0.107, p

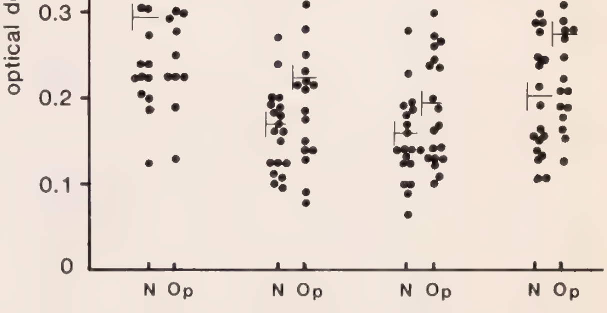


Fig. 4. Reactivity, assessed in an ELISA, of sera from patients with Graves' ophthalmopathy (Op) and normal subjects (N), against Harderian gland cytosol antigens isolated with an MCAb (peaks II and IV, Fig. 3) and control pools (I and III). Results are expressed as optical density at 410 nm. Horizontal bars represent mean (±standard error) values.

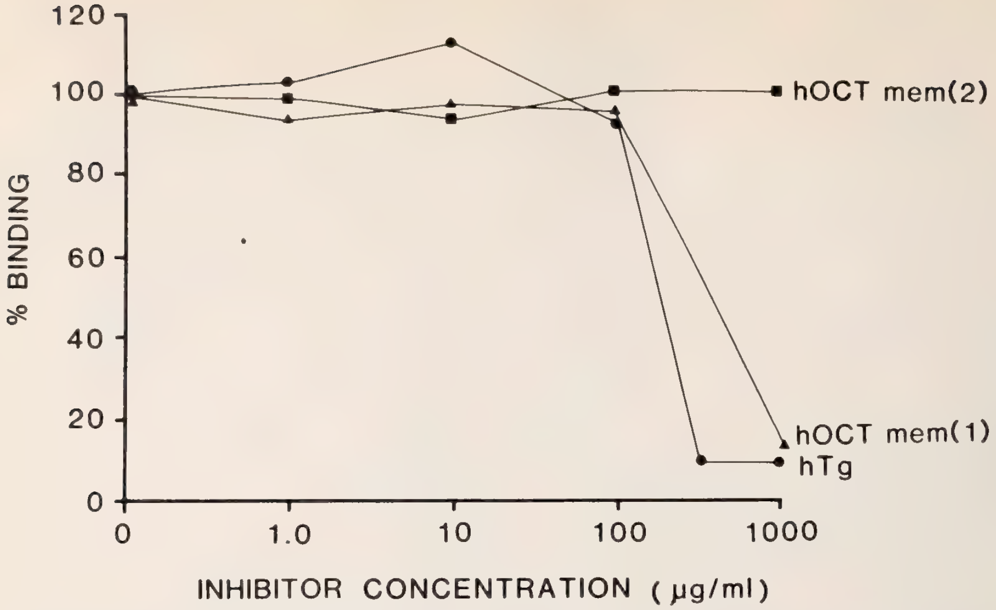
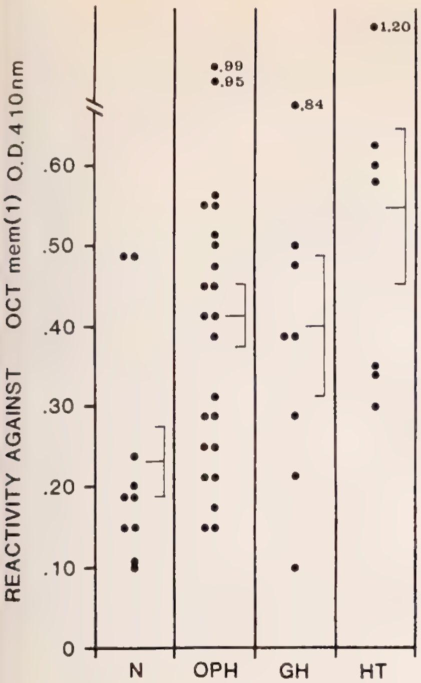


Fig. 5. Reactivity, assessed in an ELISA, of antithyroglobulin MCAB (A3) with OCT membrane after absorption, at 37°C overnight, with OCT membrane (1) and (2) and thyroglobulin (Tg). Results expressed as percentage of binding, that is, optical density with inhibitor) − (background)/(optical density without inhibitor) − (background) × 100, at increasing inhibitor concentrations.

not significant) was used as the source of OCT membrane antigen. Although mouse MCABs against other thyroid or orbital tissue antigens often showed cross-reactivity (as described above, this was usually against a ubiquitous antigen found in all tissues tested, and reaction against an antigen shared only between thyroglobulin and OCT membrane was not demonstrated.

Epstein-Barr virus-infected human cell lines and human hybridomas produced from peripheral blood lymphocytes from patients with Graves' ophthalmopathy were also tested for cross-reactivity between thyroid and orbital tissues. Of 30 EBV-infected cell lines cloned by limiting dilution from a single cell line, 20 reacted against an antigen shared between thyroglobulin, OCT membrane, lacrimal gland membrane, eye muscle membrane, and liver membrane. In no case was reaction restricted to OCT membrane and thyroglobulin. Uncloned EBV-infected cell lines showed widespread reactivity between thyroid and orbital tissue, but monoclonality of these lines has not been established. Forty fusions of EBV-infected cell lines with human myeloma cells have been performed and have been tested for cross-reactivity. Of the more than 150 hybridomas secreting antibody reactive with thyroid or orbital antigens, 16 (14%) of the 113 studied so far show reactivity against one or more shared antigens: in four cases between thyroglobulin and OCT membrane, in ten cases between thyroglobulin and one or more other orbital tissue preparations, and in two cases between thyroid membrane and OCT membrane antigens. Monoclonality of these hybridomas is being established by subcloning by limiting dilution.

Sera from patients with Graves' ophthalmopathy and autoimmune thyroid disorders were tested for reactivity with orbital connective tissue membranes. Tests were positive (optical density >0.25) in 18 of 23 patients with Graves' ophthalmopathy, in 6 of 8 patients with Graves' hyperthyroidism and no associated eye disease, in 6 of 8 patients with Hashimoto's thyroiditis and no eye disease, and in 2 of 8 normal subjects (Fig. 6). Mean (± standard error) levels of anti-OCT membrane 1 antibody in patients with ophthalmopathy (0.42 ± 0.04; $n = 23$) and Graves' hyperthyroidism without eye disease (0.40 ± 0.75, $n = 8$) were not significantly different (t tests, p not significant), but mean (± standard error) levels in patients with Hashimoto's thyroiditis without eye disease (0.54 ± 0.08, $n = 8$) were significantly greater than those in both of these groups of patients ($p < 0.05$ and $p < 0.05$, respectively). Serum levels of antibody against the OCT membrane antigen were not closely correlated with the presence, duration, or severity of the eye disease. There was, however, a close positive correlation between levels of antithyroglobulin antibody and anti-OCT membrane 1 antibody ($r = 0.85$, $p < 0.001$) (Fig. 7). It seemed



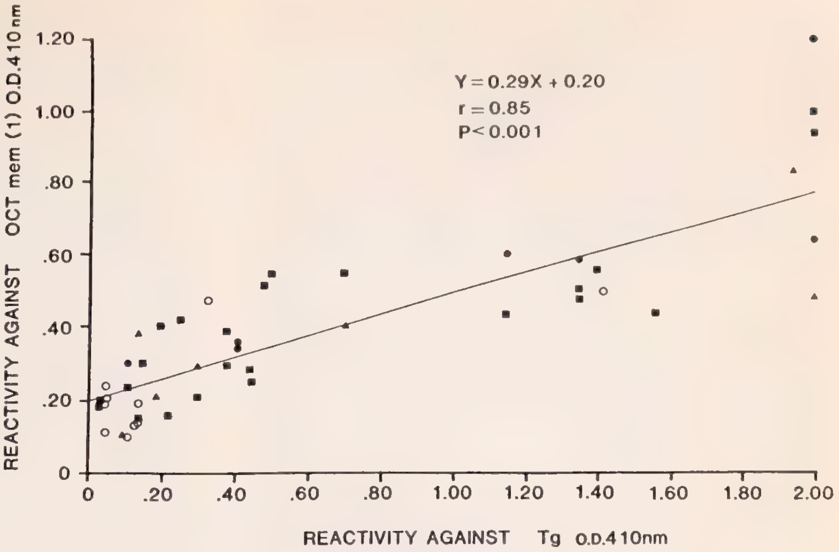


Fig. 6. Left Reactivity, assessed in an ELISA, of sera against human orbital connective issue membranes [OCTmem(1)] in patients with Graves' ophthalmopathy (OPH), Graves' hyperthyroidism without eye disease (GH), Hashimoto's thyroiditis without ophthalmopathy (HT), and normal subjects (N). Horizontal bars represent mean (±standard error) values. Results are expressed as optical density at 410 nm. Positive test was optical density > 0.25. Serum dilution was 1:100.

Fig. 7. Right Correlation between antibody activities against OCT membrane (1) and against thyroglobulin in sera from patients with Graves' ophthalmopathy (●), Graves' hyperthyroidism without eye disease (▲), Hashimoto's thyroiditis without eye disease (■), and normal subjects (○); *r* = correlation coefficient, *Y* = gradient.

likely, therefore, that the serum autoantibodies reactive with the OCT membrane antigen were antithyroglobulin. This was confirmed by absorption experiments, activity against OCT membrane being completely absorbed by preincubation with thyroglobulin, thyroid membrane, and OCT membrane, but not skin, eye muscle, or lacrimal membrane (results not shown).

Discussion

We have produced more than 200 mouse and human MCABs against human thyroid and orbital tissue antigens. A large proportion of these MCABs cross-reacted with one or more antigens, although for the human antibodies monoclonality has not yet been established. Many of these have been used in our studies of the pathogenesis of Graves' ophthalmopathy. Using mouse antibody 50-24, we were able to purify from crude human eye muscle cytosol, by sequential chromatography and affinity chromatography, a soluble antigen, S, previously shown to be an autoantigen in the majority of patients with Graves' ophthalmopathy (11). The antigen was shown to be a ganglioside, although only crude preparations were tested. Using MCABs against guinea pig Harderian gland, we isolated two soluble antigens from the cytosol fraction. When sera from patients with Graves' ophthalmopathy were tested for autoantibodies against these antigens, 20% of the patients were shown to have circulating antibodies against one of the antigens, even though, overall, there were no significant differences between patients and normal subjects for either antigen.

Eye muscle inflammation appears to be the primary abnormality in Graves' ophthalmopathy (22, 23). This may be mediated by K cells and cell-surface-directed antibody, as is the case for thyroid cell cytotoxicity (24), although there is little evidence for this at present. Kendall-Taylor et al (12) and Faryna et al (13) demonstrated antibodies against an eye muscle membrane antigen in approximately 60% of patients with eye disease, although we have not been able to confirm either of these findings (J.R. Wall et al, unpublished results). We reported antibodies against the soluble antigen S, purified in the present study with MCAB 50-24 (11), but their role has not yet been determined. The role of antibodies against Harderian gland antigens is even less certain. However, our finding of autoantibodies against an MCAB-isolated Harderian gland soluble antigen in 20% of patients with Graves' ophthalmopathy suggest that such antibodies may play a role in the pathogenesis of this disorder. Indeed, in early studies several workers (25–28) demonstrated binding of patient serum or immunoglobulin to guinea pig Harderian gland membranes. Moreover, we have induced experimental Harderian gland inflammation in guinea pigs immunized with Harderian gland extract; the inflammation was greater in hyperthyroid than euthyroid animals and was associated with serum antibodies against Harderian gland antigens (J. R. Wall et al, unpublished results). There is no evidence that human orbital tissue contains a Harderian-gland-equivalent antigen. For example, none of our three Harderian gland monoclonal antibodies cross-reacted with human orbital connective tissue.

In the main part of the present studies we used MCABs against human thyroglobulin and found an antigen-reactive site in human orbital connective tissue membranes which is shared with human thyroglobulin and against which antithyroglobulin autoantibodies from patients with autoimmune thyroid disorders, with or without Graves' ophthalmopathy, react. The OCT membrane thyroglobulin shared antigen was not native thyroglobulin deposited in the tissue (which was prepared from normal subjects). On the other hand, the reactive site is clearly an important part of the thyroglobulin antigenic site, since all but 6 of the 17 MCABs tested reacted with at least one preparation of OCT membrane tested and both the serum (auto) and monoclonal antibody activities were completely absorbed by all preparations of native thyroglobulin tested. Thus it seems likely that the OCT membrane reactive site is a small part of thyroglobulin and integral with its membrane structure. Since most MCABs and many sera also reacted with liver membranes, the shared protein must also be present in this organ.

Although this is apparently the first report of cross-reactivity against antigens shared between two target organs in autoimmune disease, there is considerable evidence for the existence of antigens shared between self-antigens and foreign (usually infectious) antigens. In particular, the finding of antigens shared between thyroid membranes and *Yersimia enterocolitica* is most interesting (29, 30). Although this is not directly relevant to the question of multiple organ-specific autoimmunity, it does suggest a possible mechanism for the development of thyroid disease in predisposed subjects.

Thus, although it is becoming clear that Graves' ophthalmopathy is an organ-specific autoimmune disorder with target autoantigens and corresponding circulating autoantibodies which may be specific or thyroid cross-reactive, much work is necessary to elucidate the nature of the antigens and the pathogenetic and clinical significance of the antibodies. Moreover, the likelihood that orbital tissue damage is mediated by T or K cell reactions needs to be studied. Although the mechanism for the association with thyroid disease is still unexplained, the finding of an autoimmune reaction against an antigen shared between orbital connective tissue and thyroglobulin raises the possibility that eye muscle autoimmunity, Hashimoto's thyroiditis, and Graves' hyperthyroidism may be linked by a common factor. Confirmation of the existence of cross-reactive antibodies, particularly if they are shown to be cytotoxic to target cells, would be of great importance in our understanding of the orbital autoimmune process.

Summary

Mouse and human monoclonal antibodies (MCABs) against thyroid and orbital tissue antigens were used to investigate aspects of the immunological basis of Graves' ophthalmopathy. Using mouse antibodies, we partially purified a human eye muscle-derived soluble autoantigen, S, and a soluble Harderian gland autoantigen. We also investigated the possible association between Graves' ophthalmopathy and autoimmune thyroid disorders suggested by autoimmune reactions against antigens shared between human orbital and thyroid tissues.

Eleven of seventeen mouse MCABs against thyroglobulin reacted, in an enzyme-linked immunosorbent assay, with an antigen in human orbital connective tissue (OCT) membranes, but not with OCT soluble fraction or with membrane or soluble fractions of human eye muscle, lacrimal gland, or skin connective tissue. The anti-OCT membrane activity was absorbed by OCT membrane and thyroglobulin but not by liver membrane or bovine serum albumin. In addition, 4 of 113 human MCABs against thyroid or orbital tissue antigens showed cross-reactivity restricted to thyroglobulin and OCT membrane. Sera from approximately 50% of patients with autoimmune thyroid disorders, with or without ophthalmopathy, also reacted with OCT membrane. Autoantibody activity against OCT membrane correlated closely with serum titers of antithyroglobulin but not with presence or severity of the eye disease. The OCT membrane reactivity was absorbed by thyroglobulin, thyroid membrane, and OCT membrane but not liver membrane,

membranes prepared from other orbital tissues, or bovine serum albumin. The OCT membrane-thyroglobulin shared antigen site was not native thyroglobulin since MCABs and serum antibodies did not react with the cytosol fraction of OCT, and the membrane antigen was not solubilizable.

Because not all patients with ophthalmopathy have detectable antithyroglobulin antibodies, and not all patients with detectable antithyroglobulin antibodies develop ophthalmopathy, it is unlikely that autoimmunity against a thyroglobulin OCT membrane shared antigen is the primary mechanism of Graves' ophthalmopathy. However, the reaction of antithyroglobulin autoantibodies with OCT membranes may be a model for autoimmune reactions against other thyroid-orbital tissue shared antigens. Human MCABs against human orbital tissue antigens are being used to characterize and purify shared orbital-specific and orbital-thyroid tissue autoantigens. These antigens and the corresponding monoclonal antibodies will provide powerful tools for further investigation, and perhaps treatment and prevention, of Graves' ophthalmopathy.

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Care of the Pregnant Woman with Tuberculosis and Her Newborn Infant: A Pediatrician's Perspective

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Abstract

Tuberculin skin testing will detect tuberculous infection or disease in many pregnant women. Tuberculous disease during gestation requires immediate treatment with isoniazid (INH) and a second drug. We favor the use of rifampin over ethambutol. The majority of skin test reactors, however, have tuberculous infection only. Despite current recommendations that INH therapy in such women be delayed until the postpartum period, there is a small but definite risk that an untreated mother will progress to active disease prior to delivery. We therefore recommend that therapy with isoniazid be initiated in pregnancy. Further investigation of the other members of the household should be undertaken immediately and any infected individuals promptly treated. These measures will decrease the likelihood that the neonate will acquire tuberculosis and simplify the management of the newborn.

Tuberculin skin testing is widely used in the United States to detect cases of tuberculosis (TB) in pregnant women. TB poses a threat to the health of a gravid woman; furthermore, it is desirable to prevent a neonate from being exposed to a mother with undiagnosed TB. Because active TB has become rare in pregnant women, screening programs primarily identify women with tuberculous infection without disease (tuberculin skin-test reactors). Traditionally, isoniazid (INH) therapy of tuberculin skin-test reactors has been delayed until after delivery of the infant (1–5). At present, this practice is being questioned.

We review here the issues relevant to the treatment of mothers with tuberculous infection and disease, and discuss the management of newborns at risk. It is our proposal that treatment of tuberculous infection be initiated during pregnancy, when medical care is regularly supervised and compliance with therapy is likely to be excellent. There is adequate time to investigate household members for infection. Separation of the neonate from the mother or family following delivery can be avoided.

Issues in Treatment

Frequency. About 1% of women of childbearing age in the United States are tuberculin skin-test reactors (6). Among certain groups of women in urban areas, a higher incidence of reactors—5%–10% or more (6, 7)—is seen. It is generally assumed that the frequency of tuberculous infection in pregnant patients is similar to that in nonpregnant women. However, current data concerning pregnant women are not available. One report which specifically studied gravid women did demonstrate a significant occurrence (0.7%) of tuberculous infection with X-ray abnormalities

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in a group of primarily white women from New York City during the early 1970s (8).

The declining incidence of TB in the United States, although encouraging, does not signify that this disease is so unusual that proper preventive measures (routine skin testing and prophylactic treatment) can be abandoned (9). The downward trend in new cases, seen in past decades, has leveled off (10), and in some populations, TB may be increasing in frequency (11, 12). Tuberculin skin testing remains important in screening pregnant women for tuberculous infection and disease, since even patients with progressive disease may not be aware of any illness at the time of diagnosis.

Preventing Transmission to Neonates. A second and equally important reason for screening pregnant women for tuberculosis is the prevention of transmission of infection to the neonate. Tuberculosis is now uncommon in young children. In 1984, only 759 cases of TB in children younger than 5 years of age were reported in this country (13). However, infants are particularly prone to severe disease. In one report, eight of twelve infants exposed to mothers with undiagnosed TB became infected, and two developed tuberculous meningitis (14). Progressive primary and acute miliary TB occur in infected infants more often than in older individuals, and without proper treatment, are fatal in well over 50% of cases (15). Such data underscore the importance of preventing young children from acquiring TB and justify the considerable effort necessary to minimize the risk of neonates being exposed to contagious mothers.

Adults with positive tuberculin skin-test reactions whose disease reactivates remain the major source of new cases of tuberculosis (16). Frequent exposure to a mother (or other household contact) with TB is the usual mode of transmission of infection to a child. Failure to consider infants at risk when a family contact is a tuberculin reactor has occasionally resulted in disastrous consequences (6, 17). The adult may be unaware of the progression of infection to TB, and the infant may become infected. Thus efforts to prevent neonatal disease must focus on the pregnant skin-test reactor (and other household members), treating infection before reactivation can occur.

All gravid women with a history of TB or in whom the tuberculin test is found to be positive should be thoroughly evaluated. Positive reactions following multiple-puncture tests should be confirmed with an intradermal Mantoux test. Careful questioning about exposure to TB, results of previous skin tests and chest radiographs, past antituberculous chemotherapy, and prior vaccination with BCG is important. With proper shielding of the abdomen, a chest x-ray during gestation is safe (18) and is valuable in assessing the extent of chest disease. Sputum and other appropriate materials can be cultured for mycobacteria.

It is essential to investigate family members and frequent contacts of reactors for occult or manifest tuberculous disease. Treating only the tuberculous mother may fail to prevent infection in the infant if the child is later exposed to another household member with active disease.

The Pregnant Woman with Tuberculous Disease

Once sputum or gastric aspirate cultures have been obtained (to confirm the diagnosis and to permit drug susceptibility studies on the isolate), the gravid woman with active TB is promptly begun on antituberculous chemotherapy, regardless of the stage of pregnancy. In this setting, the seriousness of the mother's disease, the harmful effect it may have on the pregnancy, and the risk of spread of infection to others take precedence over possible effects of the drugs upon the fetus.

Choice of Agents. Isoniazid, ethambutol, and rifampin have been carefully evaluated for toxicity in a limited number of pregnancies (19). INH has been most widely used (over 1,400 pregnancies) and to date has not been shown to be teratogenic or harmful to the fetus. Rifampin is a valuable bactericidal antituberculous agent that is widely used in nonpregnant patients. Some authors propose that only INH and ethambutol, a bacteristatic drug, be used in the gravid woman with TB (19). They are hesitant to use rifampin in pregnancy because a study of fetal and neonatal abnormalities in 442 pregnancies associated with rifampin use revealed 3 patients with limb reduction abnormalities and 13 patients with hemorrhagic tendency or bleeding (19). However, the same authors believe that rifampin should be used in severe cases of TB because INH and ethambutol may fail to cure such patients (20). The overall rate of neonatal malformations when women received rifampin or ethambutol was within the range (2%–4%) expected in pregnancies in the general population.

After weighing the effectiveness of rifampin versus its toxicity, we recommend INH plus rifampin for pregnant women with miliary, meningeal, cavitary, progressive primary, or extrapulmonary TB, even in the first trimester. For other patients, we would initially prescribe INH and ethambutol and change the ethambutol to rifampin in the last trimester. Some of the infants with bleeding tendency (21, 22) may not have received vitamin K at birth. It now seems prudent to routinely administer vitamin K to the infants of such mothers (23). Optic neuritis, known to occur in adults receiving ethambutol, might be difficult to detect in a neonate (24). Follow-up ophthalmologic evaluations of children whose mothers received ethambutol in pregnancy may be indicated.

Preventing Infection of the Infant. Separation of the infant from the mother, INH prophylaxis, and vaccination with BCG have each been proposed as methods of preventing infection in neonates whose mothers have untreated, pulmonary TB (14, 25, 26). Each approach alone is imperfect.

Separation. Fifty percent of infants who were isolated from their tuberculous mothers until the mother was sputum-negative on treatment and considered noninfectious later developed positive skin-test reactions or clinical TB (15). In some cases, transmission of TB occurred following brief postnatal contact with the mother. It is also likely that many neonates became infected upon return to their mothers and families (27). The mother may not truly have been noninfectious, or an additional undetected case of TB may have been present in the household. Separation is not necessary if the mother received effective therapy during pregnancy.

INH Prophylaxis. The use of INH prophylaxis was originally not endorsed because the safety, efficacy, and optimal dosage in infants had not been rigorously studied (14, 25–27) and because in certain cases compliance was poor (28). Since that time, a wide experience with INH in infants has shown that the drug is safe and effective (29–31).

BCG. Disease due to *Mycobacterium tuberculosis* may occur in patients previously immunized with BCG. Some authors have proposed that, following BCG vaccine, reactions to 5 TU PPD above 10–15 mm in diameter are indicative of infection with *M. tuberculosis* (15, 32–35). While this may be true, other data show that the significance of skin-test results in patients vaccinated with BCG at birth is unclear in the first year of life (32, 33). This is precisely when TB poses the greatest risk to the child born to a tuberculous mother. Protection against tuberculous infection following BCG immunization is conferred only after delayed hypersensitivity develops (about 6 weeks after vaccination). Separation from the mother during this interval has been proposed (14, 25, 26). This isolation poses a hardship upon the family.

A neonate born to a mother with untreated tuberculous disease should be isolated from mother and family members. We begin therapy in the mother (INH and rifampin) and isoniazid prophylaxis in the child (10–20 mg/kg/day). The mother may have contact with her newborn only after sputum smears are negative for tubercle bacilli and at least two weeks of chemotherapy have been completed, for she is no longer likely to be contagious (36). Other members of the household may visit with the infant if their tuberculin skin-test reaction is negative or, if their reaction is positive, only after they have been evaluated further and appropriate therapy has been initiated. We prefer to continue giving the infant INH until he or she reaches 3 months of age. If the infant's reaction to PPD is negative at that time, it is unlikely that tuberculous infection resulted from exposure to the mother in the neonatal period, and the infant's medication may be stopped. Therapy in the mother is continued. Infants with positive Mantoux tests are investigated further and treatment is continued.

When it is uncertain that the INH will be given reliably or that follow-up appointments will be kept, we agree with others (14, 26, 37) who believe that BCG vaccine should be given to the infant. The child should be kept away from the mother and other infected contacts until his or her Mantoux test becomes positive. Giving INH prophylaxis concurrent with a standard BCG vaccine (38–40) or with an INH-resistant BCG strain (38, 41) is an attractive way of preventing tuberculosis in the period before delayed hypersensitivity develops, and separation while in the hospital would not be imperative. However, INH-resistant strains are not generally available in the United States, and there is concern that INH might diminish the efficacy of standard BCG vaccines (42).

Breast Feeding. The major contraindication to breast feeding the infant of a tuberculous mother concerns the close contact of mother and child. Nursing may be initiated when the necessity for isolation has passed. The infant should receive INH prophylaxis (29). Both INH and rifampin appear in breast milk; however, the infant typically receives less than 1% of the total daily maternal dose (43).

The Pregnant Woman with Tuberculous Infection Without Disease (Skin-Test Reactors)

Quiescent infection will progress to TB in 5%–15% of tuberculin skin-test reactors in the United States (44). The incidence of progression is highest in the years following initial infection and declines subsequently. However, this risk of TB persists for the life of the reactor. Certain groups of patients who are at greatest risk for reactivation of disease have been identified: household contacts of an active case of TB, recent converters, and patients with chest x-ray abnormalities, immunosuppression, or underlying illnesses (such as diabetes). The American Thoracic Society (1, 45) has recommended INH therapy for nonpregnant tuberculin reactors in these high-risk categories, regardless of age. When no risk factors are identified, preventive therapy is recommended only for those reactors under 35 years of age, because children and young adults are more likely to have been recently infected and drug toxicity is unusual.

Women who are skin-test reactors without disease constitute the largest group of pregnant women with tuberculous infection. Specific information on the risk of reactivation of disease in pregnancy has not been reported. However, it is probable that the occurrence of TB is similar to that in comparable nonpregnant patients. Over 3% of recent tuberculin converters progress to TB during the first year after infection (16, 46, 47). Each year, reactivation occurs in 0.8% of tuberculin-positive adults with abnormal chest x-ray findings and in 0.08% of those with a negative x-ray (46, 47). If newly infected individuals are excluded, about 0.2% of reactors progress to TB each year (16).

The following classification of pregnant women with tuberculous infection is useful in determining the proper therapeutic strategy.

Women Already Adequately Treated. The gravid woman who in the past complied with recommended standards of drug treatment for tuberculous infection or disease is unlikely to have active TB or to infect her child. However, a chest radiograph should be obtained in the third trimester and compared to previous films. In instances where there is any clinical or radiological suspicion of ongoing disease, smears and cultures of sputum should be obtained. Therapy is not necessary if all studies are negative. Such women, however, require careful observation for reactivation during the remainder of pregnancy and the postpartum period. A Mantoux test in the infant should be done at two and six months of age.

Recent Converters. The pregnant patient whose skin reactivity to tuberculin has changed from negative to positive within the past two years is considered a recent converter. Due to the high risk of progressive disease, chemotherapy is begun (45). If the chest x-ray is normal or shows a healed primary complex, INH alone is considered adequate therapy; if the x-ray is abnormal, two drugs are used. Frequently, screening during pregnancy detects previously undiagnosed tuberculin reactivity in a woman who has not been tested in the recent past. We prefer to consider such patients to be recent converters, although this cannot be known with certainty.

Women with Special Conditions. As in nonpregnant patients, never-treated gravid women with positive tuberculin skin-test reactions should receive isoniazid when they are immunosuppressed or diabetic, receive steroids, or have undergone or are about to undergo extensive surgery (especially gastrectomy).

Other Tuberculin Skin-Test Reactors. A large number of pregnant women whose skin-test reaction became positive in the distant past or at an unknown time are asymptomatic and have never received antituberculous therapy. The chest x-ray shows no evidence of TB or only a "healed" Ghon complex, areas of calcification, or apical scarring. The American Thoracic Society formerly proposed that therapy of tuberculous infection be postponed until after delivery of the fetus (1). This advice was based on the belief that the risk of reactivation of quiescent infection was greatest in the postpartum period and not during pregnancy itself. These authors were also concerned about possible adverse effects of INH upon the fetus.

Recently, the ATS recommendations have been revised (45), so that objections to the use of isoniazid in pregnancy have been removed. Furthermore, the assertion that the "increased risk of tuberculosis for new mothers is during the postpartum period, not during pregnancy" has been omitted. This seems advisable, since the studies of Hedvall (48), Cromie (49), and Good (50) show that progression of infection certainly can occur during gestation. INH preventive therapy is now recommended in pregnancy if tuberculous infection was recent (45).

We and others (6) favor extending this recommendation to include treatment of all pregnant women under 35 years of age with positive skin-test reactions, unless specific contraindications exist. It is also our practice to recommend therapy for reactors with chest x-ray abnormalities (other than a healed Ghon complex or calcifications) and for those who are close contacts of active cases of TB, regardless of age. We are concerned that a small number of women who are felt to have tuberculous infection early in pregnancy may already have slowly progressive disease and that others may subsequently reactivate prior to delivery. Not only is the well-being of the mother (who in fact has active disease) in jeopardy, but her newborn is at risk as well.

Therapy with isoniazid is begun after the first trimester, when organogenesis has been completed. Any theoretical teratogenic effect of INH can thus be avoided. However, in over 400 pregnancies in which INH was taken at the time of conception and during the first trimester, no teratogenic effect has been noted (19).

Data on the hepatotoxicity of INH have shown that transient, mild liver dysfunction is common during therapy, but usually this does not necessitate stopping the drug. More severe liver injury is unusual and occurs primarily in patients with prior liver disease or advanced age. Progressive liver damage from INH is rare in patients under 20 years old and develops in up to 0.3% of those 20–34 years of age, 1.2% of those 35–49 years of age, and 2.3% of those 50–64 years of age (51, 52). Little has been published about adverse effects of INH in pregnant women. However, there is no mention of severe liver injury in two reports (53, 54) prior to the wide recognition of hepatotoxicity from INH (600 patients and 10 patients, respectively) and in one (50) subsequently (27 patients).

Treatment with INH prevents reactivation of dormant tubercle bacilli and may stabilize or improve unsuspected tuberculous disease. By beginning therapy before delivery, the care of the infant is greatly simplified.

Management of Infants Born to Mothers with Untreated Tuberculous Infection

What constitutes the proper management of infants born to never-treated skin-test reactors, when complete evaluation of the mother and family members was postponed until after delivery? Such mothers usually have tuberculous infection only; however, it seems prudent to separate the child from mother and household contacts until active disease in them is excluded. History, physical examination, tuberculin skin test, and chest x-ray, when indicated, are useful in rapidly assessing the potential of each individual to infect the neonate. A mother without a positive sputum or systemic signs, whose chest radiograph is normal, is considered to have tuberculous infection only and is allowed contact with her infant. A one-year course of isoniazid for the mother is begun. The neonate is observed without treatment and monitored for conversion of the skin-test reaction (2, 55, 56).

The physician must also evaluate family members for disease. It is often difficult to arrange for inoculation and evaluation of tuberculin skin-test reactions during the few days that the mother and infant remain in the hospital. In this brief time, the physician strives to educate and to elicit the trust and cooperation of the mother and family—who unfortunately are likely to view the physician as an adversary who is preventing them from being with the baby.

It is our belief that a simpler strategy is to eliminate the risk of TB in the infant by eradicating the disease or threat of disease in the mother and the household before the child is born. The problem of dealing with the infant on an emergency basis is then obviated. Effective chemotherapy during pregnancy greatly reduces the risk of the neonate acquiring infection. Isolation from the mother is not necessary, sparing the mother the traumatic surprise of being told she cannot hold or be near her newborn infant. Close observation of the infant is all that is required. There is no need to give the infant INH or BCG vaccine, and the skin test retains its value in the future care of the infant. When intervention first occurs after the birth of the infant, compliance with medications and follow-up examinations has been poor (28). Perhaps the heightened acceptance of medical care exhibited by many women during pregnancy, and the avoidance of situations in which the mother and family see the medical staff as antagonists, will result in better compliance.

An aggressive approach to diagnosing and treating tuberculous infection and disease in gravid women that begins during pregnancy will benefit the mother, prevent infection in the child, and greatly simplify the management of the newborn.

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Chlamydia trachomatis Infections in Infants

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Abstract

The level of chlamydial conjunctivitis and respiratory tract infection in infants was determined. Cultures for chlamydial infection were taken from 30 infants; 14 (46.7%) of these cultures were positive. Of the 21 infants tested for chlamydial conjunctivitis, 11 showed positive for *Chlamydia trachomatis*. Of the 9 infants tested for respiratory tract infection, 3 were positive for *C. trachomatis*. We conclude that chlamydial infection in infants is of greater concern than has been previously reported.

Sexually transmitted diseases in infants can be acquired from their mothers during passage through the birth canal. This has been reported for *Neisseria gonorrhoeae* (1) and *Chlamydia trachomatis* (2–4). Conjunctivitis and/or pneumonia was found in 44%–70% of infants born to mothers infected with *C. trachomatis* (2–4). In addition, Schachter et al (5) have reported recovering *C. trachomatis* from the rectum and vagina of infants born to infected mothers. Schachter's group, at San Francisco General Hospital, further showed that chlamydial infection was more prevalent among black women, most likely reflecting their socioeconomic status (3).

Previous studies have given evidence for *C. trachomatis* as the causative agent in ophthalmia neonatorum and neonatal pneumonia (6–8). Rees et al (6) isolated *C. trachomatis* in 35.0% of infants with conjunctivitis. Armstrong et al (7) reported comparable levels of chlamydial infection (29.4%) in infants with conjunctivitis. In addition, *C. trachomatis* was found to be the causative agent in as many as one-third of infants with pneumonitis (8).

Armstrong et al (7) state that chlamydial infection is the major cause of ophthalmia neonatorum in inner-city populations. This study was designed to assess the prevalence of chlamydial infection in a high-risk infant population using a highly sensitive immunofluorescent culture technique (9).

Methods

Patient Population and Sample Collection. Mount Sinai Hospital Medical Center serves a largely black and Hispanic, low-income population on the southwest side of Chicago. Culture specimens were obtained by pediatricians from 30 infants under one year of age. Swabs were taken from the eyes of 21 infants with conjunctivitis and from the throats of 9 patients with suspected respiratory tract infection. All specimens were placed immediately in a sucrose phosphate buffer (2SP) and transported to the Reproductive Microbiology Laboratory of the Department of

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Obstetrics and Gynecology. Most specimens were stored at 4°C and cultured for *C. trachomatis* within 24 hr. Specimens stored for longer periods were frozen at −70°C until culturing.

Isolation and Detection of *Chlamydia*. *Chlamydia* was isolated on cycloheximide-treated McCoy cells grown on 12-mm coverslips in 15 × 45 mm vials. Cells were pretreated with 1.0 ml diethylaminoethyldextran (30 μg/ml, DEAE-D, Sigma Co.) for 30 min immediately prior to inoculation with specimen. After pretreatment with DEAE-D, cells were inoculated with 0.2 ml of transport media containing the specimen. The vials were then centrifuged for 1 hr at 500 g (Centra-7, IEC). The inoculated McCoy cells were then incubated at 37°C for 72 hr in minimal essential media (Flow Labs) modified with HEPES (Sigma Co.), gentamycin, and vancomycin.

Chlamydia inclusions were detected using fluorescein-conjugated antibodies in an Evans' blue counterstain (Microtrak, *Chlamydia trachomatis* Culture Confirmation Test, Syva Co.). Cultures were fixed by adding 1.0 ml of absolute methanol to the vials after aspiration of the culture media. After 10 min the methanol was aspirated and cells were rinsed with deionized water. Fixed cultures were then stained with 30 μl of fluorescein-conjugated antibodies and incubated for 20–30 min at 37°C. Cells were then rinsed with deionized water to remove excess antibody, and coverslips were removed from the vials and mounted. Coverslips were then examined at 200×, 400×, and 1,000× for characteristic inclusion, elementary, and reticulate bodies.

Results

Specimens from 30 infants with conjunctivitis or suspected respiratory tract infection were received by the laboratory. These infants ranged from one day to nine months of age. Of these, 21 were diagnosed as having conjunctivitis (age range: one day to nine months) and 9 were diagnosed as having respiratory tract infection (age range: 24 days to eight months). The infants with suspected respiratory tract infection included 5 with pneumonia, 1 with bronchitis, and 3 with infection of the throat.

Of the 21 infants with conjunctivitis, 11 showed positive cultures for *C. trachomatis* (52.4%; Table). This included a set of one-month-old twins. The age range of infants with chlamydial conjunctivitis was one day to nine months.

Positive chlamydial cultures were found in 3 of the 9 infants with suspected respiratory tract infection (Table). These 3 infants were 24 days, 3 months, and 8 months.

TABLE

Prevalence of Chlamydia trachomatis *in Infants with Conjunctivitis and Respiratory Tract Infection*

	Total *n*	No. positive	% positive
Conjunctivitis	21	11	52.4
Pneumonia	9	3	33.3
Total	30	14	46.7

Discussion

C. trachomatis is probably the most prevalent sexually transmitted disease (10). Furthermore, the prevalence of chlamydial infection in pregnant women has been reported to range from 2% to 12.7% (2–4, 11). Therefore, a large number of infants are exposed to the infectious agent. In addition, it has been shown that 44%–70% of infants born to infected mothers will develop chlamydial infections (3, 4).

A large number of conjunctivitis and respiratory tract infections may be caused by *C. trachomatis* (6–8). The prevalence rate of chlamydial infection in infants with respiratory infections found in our data (33%) is higher than the generally accepted prevalence rate (10). In addition, our data on prevalence rate of chlamydial conjunctivitis (53.4%) is higher than that reported elsewhere in the literature (6, 7).

The discrepancy in prevalence rates may stem from multiple factors. First, this study employed different techniques for the isolation and detection of *C. trachomatis* than those used in previous studies (6–8). Our group used polycation (DEAE-D) pretreated cells for culture. It has been shown that DEAE-D enhances the attachment of *C. trachomatis* to cells and may facilitate the internalization of *Chlamydia* (12). We also used an immunofluorescent technique which has only recently become commercially available. Detection of *C. trachomatis* in cell culture with immunofluorescence has been shown to be highly sensitive (9).

A second factor that may cause the high prevalence rates in our study is that the endemic level of *C. trachomatis* in our patient population may be higher. It has been established that black and low-income groups have a higher level of chlamydial infection (3, 4, 11). In addition, preliminary data from incidence studies in the gynecologic and obstetric population of Mount Sinai Hospital Medical Center, Chicago, indicate a higher incidence than reported by other U.S. centers (13).

Finally, since our study population, especially of infants with respiratory infection, is small, it may not constitute a generalizable sample of our patient population. Our data, however, do suggest that *Chlamydia* infection rates may be underestimated when standard culture methods are used. Furthermore, our data suggest that *C. trachomatis* should be strongly considered when attempting to diagnose the causative agent in infant conjunctivitis.

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Outpatient versus Inpatient Cone Biopsy of the Cervix

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Abstract

A fifteen-month prospective study was conducted to analyze the complication rate and cost effectiveness of performing cone biopsies of the cervix as ambulatory procedures. The rate of postoperative complications was found to be low, and the financial savings of the outpatient over the inpatient cone biopsy were substantial.

It has become dramatically apparent during the past decade that the economic resources available to meet the demands of health care are limited. Public as well as governmental pressure has resulted in an analysis of many of the procedures and therapies utilized in the health care system (1).

Many gynecologic procedures such as tubal ligations, dilatations and curettage, and diagnostic laparoscopies that were routinely performed as inpatient surgery only a few years ago are now commonly performed on an outpatient basis. In many states, governmental regulations tied to state or federal aid programs restrict inpatient procedures to encourage outpatient treatments.

In the United States, 82,000 cone biopsies are performed annually (2). Because cone biopsy of the cervix has been routinely performed as an inpatient procedure, our department conducted a fifteen-month prospective study to evaluate the complication rate and cost effectiveness of performing cone biopsies of the cervix as an outpatient procedure.

Method. A fifteen-month prospective study was conducted from March 1, 1982 to July 1, 1983 and included 39 patients who had conization of the cervix performed as an outpatient procedure. The median age of these patients was 25 years; range, 20–64 years. Preoperatively, a complete history and physical examination together with blood studies (complete blood count, type and screen, urinalysis, and urine pregnancy test) were done. The procedure was explained to the patient and possible postoperative complications were emphasized. All conizations were performed with the patient under general anesthesia and a modification of the technique of VillaSanta was employed (3, 4). The technique involves using the same suture that ligates the descending branch of the uterine artery bilaterally to purse-string the anterior and posterior cervical lips for hemostasis. After the procedure, the patient was observed in the recovery room for two hours and then in a Step Down Unit until she was stable and alert. The patient was then instructed to return to the gynecology clinic in one week from the time of discharge. The only medications prescribed at discharge were analgesics. At postoperative examinations one week and eight weeks following the surgery, the cervix was routinely sounded to prevent cervical stenosis.

Results. The mean blood loss during the procedure was 75 ccs with a range of 25 to 200 ccs. No significant perioperative or immediate postoperative complications were encountered. Twelve hours after one patient was discharged, she had

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bleeding that required some additional suture ligations to obtain hemostasis. No complications occurred later than 24 hours after surgery, and, to date, no patient has developed cervical stenosis.

The cost for operating room and anesthesia time at our institution is the same for inpatients and outpatients (Table). The average length of hospitalization for inpatient conization of the cervix had been two days prior to our study; thus a savings of $714.00 per patient in room charges alone is realized. Further savings of approximately $30.00 per patient result from a decrease in pharmacy and laboratory charges. The only additional cost for outpatient treatment is the charge of $34.50 per hour for observation in the Step Down Unit; the majority of patients were observed for only one hour in this unit prior to discharge. Thus, the total savings amounted to approximately $700.00 per patient.

Discussion. Cone biopsy of the cervix is a frequently performed gynecologic procedure. Traditionally, it has been performed as an inpatient procedure and generally has involved a minimum of two days of hospitalization. Clinicians have been hesitant to perform cone biopsy as an outpatient procedure because of the presumed frequent complications, hemorrhage and infection. However, using the modified technique of VillaSanta together with proper patient selection and education, our study, though limited, proved that this ambulatory procedure approach represents an effective method of conization with minimal complications and substantial financial savings. However, some patients may not be suitable candidates for outpatient surgery, primarily because they have potentially complicating medical problems.

Further reduction in cost could be made by performing the procedure with the patient under paracervical block, thus diminishing both costs of anesthesia and risks to the patient. McDonell and Stenger (5) demonstrated that patients tolerate the procedure well under local infiltration. In a study of tubal ligations as ambulatory procedures we were also able to demonstrate that ambulatory surgery results in a significant diminution of the demand for analgesics (6).

A nationwide average of 82,000 cone biopsies are performed as inpatient procedures annually. If cervical cone biopsies were performed as outpatient procedures, the savings would amount to tens of millions of dollars annually.

TABLE

Inpatient versus Outpatient Cost for Cone Biopsy of the Cervix

Charge	Inpatient Rate	Outpatient Rate
Room	$372 per day	None
Anesthesia	$300 <90 min	$300 <90 min
Operating Room	$523 per hour	$523 per hour
Recovery Room	$69 per hour	$69 per hour
Step Down Unit	None	$34.50 per hour
CBC (postoperative)	$24	None
Analgesics	$0.75 per tablet	$0.25 per tablet

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Update on Hypnotics for Insomnia in Clinical Practice:
I. Hypnotics

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Abstract

In a given year 60 million adults in the United States will have trouble sleeping, and 30 million hypnotic prescriptions will be written. This article updates conclusions and recommendations on hypnotic use (Part I). Related questions of evaluation and management of insomnia will be discussed in Part II. Because many issues remain to be resolved by research data, physicians must not be overly committed to particular hypnotics or procedures. The present enthusiasm for benzodiazepines should be moderated; new information shows them (and this includes the two most recent hypnotics to enter the market, triazolam and temazepam) to be far from ideal.

Each year, 60 million adults in the United States (or up to 38% of all persons over 18) will suffer from the sleep problem of insomnia (1). Perhaps 42.5% of all adults have at some time had more than an occasional night of insomnia (2), and essentially everyone has experienced some trouble sleeping. Nineteen percent of all physicians' patients complain of insomnia (3), the commonest reason people seek medical help (after headaches and colds) (4). Among specialists, psychiatrists have the highest percentage of patients complaining of insomnia (33.7%) (3).

In trying to give relief, U.S. physicians have given nearly 30,000,000 prescriptions for hypnotics per year (4). This is a large number, yet only 10%–20% of insomnia sufferers receive relief using prescribed hypnotics (1). Although essentially all clinically oriented physicians prescribe hypnotics, psychiatrists proportionally prescribe hypnotics the most; they receive about 3% of all office visits to physicians, yet prescribe 23% of all hypnotics (1).

The most comprehensive of recent reviews of the clinical use of hypnotics was issued in 1979 by the Institute of Medicine, supported by the U.S. president and the Department of Health, Education, and Welfare (1). This report recommended reviews of hypnotics every 2–3 years (because of the rapid development in sleep research) and vigorous educational programs promulgating any new findings by medical societies and governments. The most recent book on sleeping pills came out in 1980 (5). The Council on Scientific Affairs of the American Medical Association surveyed hypnotics briefly in a 1981 report (6). Finally, a large panel of experts convened in November 1983 to discuss hypnotics. This article uses all these resources and others to provide an integrated view and recommendations on hypnotics in clinical practice, including emphasis on the hypnotic that has been marketed since 1982—temazepam (Restoril)—and the one marketed since 1983—triazolam (Halcion).

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Hypnotics

Hypnotics are substances used primarily to induce a sleeplike state; I discuss here only those approved by the U.S. Food and Drug Administration as safe and effective prescription drugs for sleeping problems and medical conditions needing restful sleep.

Over millennia, the popularity of each of the many types of sleeping potions available has waxed and waned depending on fashion, advertisements, regulations, and other factors in each generation. One example is chloral hydrate, the oldest synthesized hypnotic; it was in general use (and significantly abused) in the late 1800s, then lost favor when barbiturates first appeared in the early 1900s. In the last 30 years, chloral hydrate has increasingly been prescribed, and although its popularity as an abused ("recreational") drug has not returned to the levels of the 1800s (1), the possibility remains (despite its listing by the federal government as having minimal abuse potential). The most popular hypnotic during the last five years in the United States has been flurazepam, over 50% of all hypnotic prescriptions (1).

Over the last 25 years, the most significant advances in our understanding of insomnia and hypnotics have derived from the research of sleep laboratories. These studies have delineated rapid eye movement (REM) sleep, the stage of extensive dreaming, and the four non-REM stages of sleep (1). Sleep laboratory studies have provided our first electroencephalographic (EEG) and other physiologic documentation of the effect of hypnotics on the percentages and varieties of sleep components. In recent years, sleep laboratory data seem to have replaced rather than supplemented other research. This is unfortunate because most sleep laboratory research is based on a highly select, tiny fraction of the huge numbers of people who suffer from sleep problems. Most sleep laboratory studies of hypnotics involve only 5–10, by and large poorly described, subjects (7). Thus, sleep research is still incomplete and does not answer many of our clinical questions about hypnotics (1); it must be supplemented by subjective measures, including daytime observations (8). Subjectivity is an important issue; the complaint of insomnia is subjective, and the physician prescribes treatment based on the subjective report of the patient.

Currently, in the United States, about 6% of adults (9–10 million a year) use a prescribed hypnotic. One-quarter of these users will take sleeping pills nightly for two or more months (1). Almost all ages and types of people use them. For examples, 2% of Harvard medical faculty use a hypnotic more than three times a week (9), and 3% of those aged 18–29 receive prescriptions yearly (10).

There are seven classes of hypnotics; these contain 16 simple hypnotics in recent use in the United States, which are listed in *Physicians' Desk Reference* (PDR) (11), the *Modern Drug Encyclopedia* (12), or both, as acceptably safe and effective (Table 1).

Safety Issues and Side Effects

The research weighing benefits against side effects of hypnotics is often conflicting. Any one of the hypnotics in use in routine clinical practice may or may not appear to be much different in overall ratio of benefits to side effects from another. Nonetheless, each drug has its own unique profile of attributes. For example, barbiturates are believed to cause a higher incidence of delirium in the elderly; hence they are rarely given to patients with organic mental disorder (4), despite the lack of research to prove this belief (1). At the other extreme, methaqualone may have the highest benefit-risk ratio for the elderly (1, 13).

Contraindications. All hypnotics are contraindicated in hypersensitivity, pregnancy, sleep apnea, suicidal intent of nonhospitalized persons, maintenance of addiction, liability to emergency on-call duty during a sleep period, or marked hepatic or renal impairment (these two organs eliminate all hypnotics). Certain hypnotics have additional contraindications. Some barbiturates are contraindicated in significant respiratory impairment, pain, and previous addictions (11). Furthermore, barbiturates precipitate attacks of porphyria, as do piperedinediones, trichlorethanols, and ethchlorvynol. Only chloral hydrate should not be prescribed in gastritis (11), because it causes gastric irritation.

Thus, methaqualone, benzodiazepines, and ethinamate have the fewest contraindications.

In many medical conditions, barbiturates, glutethimide, and chloral hydrate probably are worrisome because they stimulate hepatic enzymes, which will cause quicker metabolization of anticoagulants, corticosteroids, calciferol, doxycycline, griseofulvin, phenytoin, valproate, and some antidepressants.

Interactions. A hypnotic can impair a patient's working performance, particularly when taken

TABLE 1

Estimated Suitability of Hypnotics in Various Conditions

	Barbiturates						Bzdp			Piped		Trichlor		Eth-chlv	Eth-inmt	Quinaz
	Amo	Apro	Hexo	Pento	Seco	Talb	Flur	Tema	Triaz	Glut	Meth	Chl	Tri			Methq
Insomnia																
Mild	3	3	3	1	2	3	1	2	3	3	3	3	3	3	3	3
Severe transient	3	3	2	1	3	3	2	2	3	2	2	4	4	2	2	4
Chronic psycho-physiologic	2	2	2	1	2	2	3	1	1	1	1	3	3	1	1	3
Initial	3	3	4	2	3	3	2	X	3	2	4	3	3	2	4	3
Terminal or middle	3	3	1	2	2	3	2	2	1	2	1	3	3	4	1	4
Suicidal	(1)	(1)	(1)	(1)	(1)	(1)	(2)	(2)	(3)	(2)	(2)	(1)	(1)	(2)	(2)	(2)
Dyspnea or pain	X	1	1	(1)	X	1	3	3	3	3	3	3	3	3	3	3
Elderly	1	1	1	1	1	1	1	2	3	2	4	2	3	3	4	3
Medical problems	1	1	1	1	1	1	2	3	3	1	3	2	2–3	3	3	3
Gastritis	4	4	4	4	4	4	4	4	4	4	4	X	3	4	4	4
Porphyria	X	X	X	X	X	X	3	3	3	X	X	X	X	X	4	4
Alcohol use																
> twice/wk	2	1	2	1	1	2	1	1	1	2	3	2	3	2–3	3	2–3
Abuse	(1)	(2)	(2)	(1)	(1)	(2)	(1)	(1)	(1)	(2)	(2)	(2)	(2)	(2)	(4)	(2)
Drug abuse probable	(1)	(2)	(2)	(1)	(1)	(2)	(2)	(4)	(3)	(2)	(2)	(2)	(2)	(2)	(4)	(2)

Numbers indicate acceptability in ascending order (1 is least acceptable). Parentheses indicate special procedures (for example, hospitalization) needed.

X = not recommended or contraindicated.

Barbiturates
amobarbital (Amytal)
aprobarbital (Alurate)
hexobarbital (Sombulex)
pentobarbital (Nembutal)
secobarbital (Seconal)
talbutal (Lotusate)

Benzodiazepines
flurazepam (Dalmane)
temazepam (Restoril)
triazolam (Halcion)

Piperedinediones
glutethimide (Doriden)
methyprylon (Noludar)
Trichlorethanol derivatives
chloral hydrate (Notec)
triclofos sodium (Triclos)

Tertiary carbinol
ethchlorvynol (Placidyl)
Urethane
ethinamate (Valmid)
Quinazolinone
methaqualone (Quaalude)

with alcohol. Of great concern is the research that shows that benzodiazepines have a particularly intense additive effect with alcohol the day following nighttime use; the combination impairs performance and memory and may cause anxiety (1, 14). This makes prescription of benzodiazepines particularly dangerous, when alcohol use is so prevalent (despite cautionary advice that the two should not be mixed). On the other hand, at least some research indicates that methaqualone has not shown this type of interaction (1).

Thus, the hypnotics that seem freest of worry about interaction with other substances are methyprylon, ethchlorvynol, ethinamate, and methaqualone.

Overdoses and Suicide. Suicide and accidental poisoning occur with all hypnotics. Which hypnotic is the least lethal? Flurazepam has the highest therapeutic index (ratio of lethal to therapeutic dose) among hypnotics. Nonetheless, there is great controversy as to whether the safety in overdose of benzodiazepines is truly greater than with other hypnotics. To resolve this debate requires going beyond the laboratory determination of a hypnotic's therapeutic index to the overdose situation in real life. For example, barbiturates (along with chloral hydrate) have the lowest therapeutic indices—ten (1). Therefore, one would expect that if the number of barbiturate prescriptions were fewer, the total overdoses would fall in number too, since most of the remaining hypnotics have higher therapeutic indices. However, when the number of barbiturate prescriptions dropped 66% from 1973 to 1980 (15), the suicide rate from all drugs remained the same (1, 6): benzodiazepine suicides increased because use of benzodiazepines increased, often in combination with other substances such as alcohol, and the combination proved lethal (16). Persons determined to die will somehow discover these combinations. Furthermore, flurazepam actually heads the list of hypnotic drugs responsible for acute drug cases seen in hospital emergency rooms; use of benzodiazepines is also more likely to be associated with a suicide motive than use of nonbenzodiazepine hypnotics (17).

Finally, not all benzodiazepines seem to have a high therapeutic ratio; for example, triazolam seems to cause serious symptoms in doses above 1 mg, only 2 times the normal dose (18).

In conclusion, no hypnotic is clearly safer than another in overdoses as these occur in real life. Discussion of differing suicide risks and therapeutic indices may be a meaningless academic exercise, since the vast majority of patients do not overdose, and patients truly intent on suicide find a way despite precautions.

Sleep Physiology. Ideally a hypnotic should not alter the normal physiology of sleep; unfortunately, the effectiveness of all hypnotics is directly related to the degree they do change the five sleep states measured by the EEG. All hypnotics suppress REM sleep in direct proportion to the size of the dose. For example, barbiturates are among the stronger REM suppressors, benzodiazepines among the weaker (1). On the other hand, benzodiazepines are perhaps the strongest suppressors of stage 4 non-REM sleep, and contrary to common belief, flurazepam distorts the sleep EEG overall more than barbiturates do (19). Generally, the nonbarbiturate, nonbenzodiazepine hypnotics change various sleep stages to an intermediate degree. Lamentably, the significance of these changes is generally unknown, although it is believed that stage 4 is associated with physical recuperation.

Half-Life and Daytime Effects. Another unwanted aspect of hypnotics is hangover (carryover of sedation into the daytime) and related additive effects with other substances affecting the central nervous system. Ideally, the effect of a hypnotic should disappear after the sleep period ends—and the drug (and its metabolites) should not accumulate, even when it is used nightly. In this regard, flurazepam is the worst, since it has the longest elimination half-life of all hypnotics, followed by pentobarbital and then secobarbital. Indeed, by the second week of use, flurazepam blood accumulation is 4–6 times greater than on the first morning (1). By contrast, blood levels of amobarbital, glutethimide, and methaqualone are not significantly higher on the fourteenth day than on the first day (1). Secobarbital also produces less hangover than flurazepam (20).

Factors that decrease drug presence and effect include shorter elimination half-life, faster tolerance development, and greater redistribution to tissues outside the central nervous system (21). There are, however, some further complexities. Despite a half-life of less than 5 hr, triazolam does have daytime residua (22). Some researchers indicate that the three hypnotic benzodiazepines are about equal in side effects (8), but others report flurazepam entails more hangover than triazolam (23, 24). Unfortunately, most research also neglects the fact that not only hypnotic effect, but also daytime alertness and reinvigoration are sought (24); so research does not yet allow clear resolution of these contradictions.

At least one report recommends use of the short-half-life hypnotics for the elderly, whose elimination of drugs tends to be prolonged (1). Hypnotics with half-lives under 5 hr are hexobarbital (5 hr), triazolam (2.5 hr), methyprylon (4 hr), and ethinamate (3 hr). Most hypnotics have half-lives of 5–24 hr: amobarbital (18 hr), aprobarbital (24 hr), talbutal (7 hr), temazepam (10.5 hr), glutethimide (11 hr), trichlorethanols (10 hr), ethchlorvynol (18 hr), and methaqualone (19 hr). The hypnotics with the longest half-lives are secobarbital (29 hr), pentobarbital (42 hr), and flurazepam (the longest half-life, 47–100 hr).

Memory Deficits. All the benzodiazepine hypnotics cause memory deficits which are potentially dangerous (25); triazolam seems to cause the most (18). We do not know whether other hypnotics also produce anterograde amnesia.

Falling Blood Levels. A variety of undesirable phenomena are related to falling blood levels of the hypnotics, including worsening of symptoms over baseline (rebound phenomenon), exaggeration of certain features, or new symptoms. Recent reports indicate that a physical withdrawal syndrome can occur and peak as late as two weeks after withdrawal from benzodiazepines given in normal doses over weeks to months; rebound phenomena do occur with flurazepam (26–30). Hypnotics with shorter half-lives tend to have stronger rebound (8, 18) and benzodiazepines with short half-lives have worse rebound than nonbenzodiazepines (31). Recent reports also indicate types of rebound that may be uniquely associated with termination (or intermittent use) after seven days (or longer) of average doses of benzodiazepine hypnotics with half-lives less than 30 hr (18). These drawbacks include "rebound insomnia" (8, 18, 31), "daytime rebound anxiety" (anxiety the day after a dose) (18, 31, 32), and "early-morning insomnia" (31, 32). Thus, an intolerable price may be paid for theoretically less hangover when using short-acting benzodiazepines. By contrast, "drug withdrawal insomnia" and "REM rebound" appear after multiple doses nightly for 1–10 years of some nonbenzodiazepine hypnotics (18, 33).

Dependence and Abuse. In practice, dependence on benzodiazepines develops about as much as on barbiturates and other hypnotics (5, 34, 35). In regard to physical addiction, 3–5 times the average dose of barbiturates, benzodiazepines, and most other drugs taken nightly for one month may result in withdrawal seizures; by contrast, methaqualone takes 7 times its hypnotic dose for one month to do so (1). All hypnotics used in such high amounts can cause physical withdrawal phenomena, including seizures and psychoses.

Physicians are concerned about nonmedical/recreational use of drugs; however, "many physicians do not routinely screen their patients for substance abuse problems, and some even seem reluctant to diagnose drug dependence until presented with irrefutable evidence in the form of an acute toxic drug reaction or withdrawal syndrome" (36). Perhaps they are aware that it is impossible to prevent the addict from duping a physician, "no matter how ethical and alert" (36). Even so, "physician contribution to drug taking represents only a fraction of the consumer market" (37).

The Council on Scientific Affairs and the National Institute on Drug Abuse have stated that there should be no attempt to stop physicians from appropriately prescribing scheduled medication, because even the elimination of prescribed scheduled medications would not affect the availability of drugs for nonmedical use or the root causes of drug abuse (6, 38). Nevertheless, physicians need to be aware of where hypnotics stand in the drug-abuse spectrum since there are drug abusers among general medical patients (36).

The substances that are most highly abused and cause the greatest amount of morbidity and mortality in the entire population are alcohol (used by about 85% of the population) and nicotine/cigarettes (used by about 20%). Following alcohol, in descending order of frequency of nonmedical use, are marijuana, hallucinogens and phencyclidines, cocaine, amphetamines, Schedule II barbiturates, inhalants, opiates, and many others (36, 39, 40). Cocaine use is currently "fashionable," and the percentage of adult users is increasing faster than for any other substance (39). Nevertheless, the nonmedical use of hypnotics, other than schedule II barbiturates, has occurred in less than 1% of the population, despite the possible "desirability" of these substances.

Indeed, all hypnotics are used nonmedically by persons seeking out drug euphoria and similar effects. Which hypnotics are most abused? We still have no scientific answer, but the most recognized system of ranking substances by potential for abuse (the federal Controlled Substances Act of 1970) indicates five levels (schedules) of abuse potential (36); drugs in "Schedule I" are said to have the highest abuse potential. Although this is the most official system, it may rank inconsistently, be outdated, conflict with some research (17, 41), and be incomplete. For example, the federal schedule ranks heroin in schedule I but not alcohol, notwithstanding the following facts:

- alcohol has as much abuse potential as pentobarbital (which is in Schedule II) (17, 41);
- alcohol is the most common self-medication for insomnia (48);
- alcohol is the most serious sedative-hypnotic problem in the world today.

The 1970 act ranks all the barbiturates in Schedules II or III, methaqualone in Schedule II, piperedinediones in III, and all the other hypnotics in Schedule IV (11).

Physicians should be aware of determinants of abuse of a particular drug in order to help them understand and identify drug abusers. The prevalence of nonmedical use of any substance depends on its pharmacology, availability, and other factors. For example, for abusers, flurazepam produces a relatively low level of desired psychic effects, yet it is available. In one study flurazepam was used nonmedically by clinic populations of severe drug abusers more than glutethimide, methyprylon, and others (42); another benzodiazepine (diazepam) was the single substance most abused—more than any other stimulant or depressant (42).

Pharmacologically, abuse of a drug tends to be greater when the drug has a shorter half-life (31), more rapid onset of effect, and greater tendency to produce disinhibition and euphoria.

Although benzodiazepine hypnotics are all ranked in Schedule IV, the known pharmacology of the new hypnotics triazolam and temazepam should make us doubt this designation. Triazolam has the shortest half-life (2–4 hr) of all 16 hypnotics (the half-life of flurazepam can be as long as 100 hr; diazepam as long as 96 hr). Also, the onset and peak effect of triazolam by mouth are about average for hypnotics. Thus, triazolam would seem to be more desirable to abusers than flurazepam. The half-life of temazepam, 8–12 hr, is shorter than that of secobarbital and amobarbital, but temazepam is the slowest of all hypnotics to take effect. Such pluses and minuses for abuse seem to cancel each other out. The larger question is whether the two new benzodiazepines produce more euphoria for the abuser. Only careful research will tell.

Clinical Choices. When after cautious evaluation the physician decides a hypnotic is necessary for a known drug abuser, he or she might prescribe a few doses at a time of temazepam or ethinamate. For the average nonabuser patient, the characteristics of the hypnotic other than potential for "recreational" use are the most important. The vast majority of patients are not abusers (36) and should not be penalized on account of the few who are (38).

Efficacy

No one can say for sure which hypnotic is most effective since no careful, controlled and replicated research comparing all the hypnotics subjectively and objectively has been done (1, 43). Nonetheless, hints to such comparisons exist. The U.S. Food and Drug Administration has approved all 16 hypnotics as safe and effective; however, at least one researcher believes that temazepam and triazolam are not effective after two weeks and are in some ways worse and overall no better than, flurazepam (18).

Falling Asleep. One of the most common symptoms for which hypnotics are prescribed is trouble falling asleep. For this problem temazepam, in the hard gelatin capsule preparation used in the United States, is of little help because it is the only hypnotic with such a long time to peak effect (2–3 hr) that it does not reduce initial insomnia. Glutethimide and ethchlorvynol are not much better (2 hr). The remaining hypnotics generally have satisfactorily short times to peak effect (less than 2 hr) after a single oral dose.

Awakenings. When the insomnia involves frequent or extended awakenings, the hypnotics with shorter half-lives (hexobarbital, triazolam, temazepam, glutethimide, methyprylon, trichlorethanols, ethinamate) would do worse than those with somewhat longer half-lives (talbutal, ethchlorvynol, methaqualone).

Acute Insomnia. The number of days needed to reach maximum effectiveness is important, especially when suffering is great. Flurazepam and pentobarbital take several days to reach maximum effectiveness, based on the accumulation that has to occur because of their long half-lives. Thus, these hypnotics are wrong for an acute severe insomnia (1), where immediate relief is desired on humanitarian grounds. It is also important to prevent the emotional trauma from leading to an episode of severe insomnia, which could cause conditioned insomnia that could go on for years (44). Certain barbiturates may be superior to flurazepam in short-term use (1).

Chronic Insomnia. There is considerable disagreement about efficacy and development of tolerance of hypnotics when they are used continuously for more than one to two weeks. Non-sleep-laboratory research on thousands of patients indicates that barbiturates and other hypnotics can be effective for years (1, 45, 46). On the other hand, a handful of sleep laboratory studies are repeatedly misquoted to opposite effect. These laboratory studies used incompletely described, highly select subjects who were either normals or chronic insomniacs—although most sleep-disorder patients have transient insomnia (1, 7, 33, 47–49). The sleep laboratory reports tend to be misquoted repeatedly as conclusive indication that, of the 16 hypnotics, only flurazepam is effective on a nightly basis for more than 3 weeks or so. In the most frequently quoted experiment (48), secobarbital, triazolam, flurazepam, glutethimide, ethchlorvynol, methaqualone were studied; each drug was given nightly to only 4 chronic insomniacs (all incompletely matched and described) for two weeks. Only glutethimide lost all effect to decrease the percent of total wake time. Secobarbital, triazolam and ethchlorvynol lost most of their effectiveness. However, methaqualone decreased in effectiveness by less than one-third, least of any drug after flurazepam. Even flurazepam has proved to have a tendency to lose effectiveness in two of the three sleep laboratory studies in which it was given over 4 weeks (49, 50). A few studies such as these can hardly be conclusive (1), yet they have influenced some manufacturers to indicate in *Physicians' Desk Reference* that glutethimide, methyprylon, ethchlorvynol, and ethinamate should be given only 7 days continuously, and probably then skipped for a week before restarting if insomnia persists (11). Barbiturates (11) and the new benzodiazepines are mentioned as losing most of their effectiveness after 14 days, which suggests that flurazepam, chloral hydrate, and methaqualone are probably more effective over longer periods. The AMA Council on Scientific Affairs (6) and a previous panel (38) take a midway stance: A prescription for any hypnotic should be sufficient only for up to 6 weeks of nightly use.

Finally, all hypnotics might prove to be as persistent in effect as flurazepam if dosage and timing were calculated to parallel the high blood levels that result from the usual nightly dosage of flurazepam. Each hypnotic would have to produce a blood level that gradually rises over two weeks to a level 6 times that of the first morning and maintain that level for another two weeks. Thus, using nonflurazepam hypnotics, one might have to keep doubling the dosage every 1–4 days to equal the pattern of persistence of flurazepam.

Although most patients take hypnotics intermittently, no research has tested the effectiveness of this type of regimen.

Clinical Choices. No one hypnotic seems intrinsically superior overall nor does any single agent appear to be the "drug of choice" for the entire insomnia population (1, 5, 6). Table 1 suggests which hypnotics may be more recommendable in certain circumstances.

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Identification and Treatment of Alcoholics Who Use Opiates

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Abstract

To analyze differences among alcoholics, we studied the characteristics of 621 patients admitted to hospital for withdrawal from alcohol in the period October 1979–March 1980. Patients were classified either as using opiates and "other drugs," using other, nonopiate drugs only, or using no drugs other than alcohol ("pure" alcoholics). The group of alcoholics using "other drugs" was similar to the "pure" alcoholics. The opiate-using alcoholics tended to be younger, had been drinking fewer years, and required less phenobarbital for their withdrawal; their liver function was more impaired. The group of alcoholics who use opiates should be distinguished as clearly as possible from "pure" and "other-drug" alcoholics, since the course of their illness seems to differ, and they may require different treatment approaches.

It has become apparent that many alcoholics abuse other drugs and that alcoholism among drug abusers is a serious problem. Freed (1) in his 1973 review concluded that 20% of addicted individuals used both alcohol and other addicting drugs, and that this pattern is more common in younger individuals. Carroll (2) et al in their review found that as many as 60%–80% of alcoholics abused other drugs and again that this pattern is more common in person under age 40. Other investigators (3–5) have reported similar findings with different rates. Ashley et al (6) compared two groups of alcoholics, a "mixed" drug-abusing group and "pure" alcoholics. They found the "mixed" group to be younger, more isolated, and more disaffiliated than the "pure" alcoholics. The "mixed" alcoholics were sicker medically, with more neurologic, genitourinary, respiratory, locomotor, and traumatic disorders. In addition, malnutrition, epilepsy, gastritis, tuberculosis, gonorrhea, and peptic ulcers were more common in the "mixed" group. The "pure" alcoholics had more cardiovascular illness and cirrhosis.

We examined a number of characteristics of patients admitted for withdrawal from alcohol, under three classifications: I, using opiates and other drugs; II, using other nonopiate drugs only; III, using no drugs other than alcohol. The group of nonopiate other drugs includes amphetamines, benzodiazepines, cocaine, methaqualone, and ethclorvinol. None of the patients required treatment for these drugs other than the phenobarbital given for alcohol withdrawal. The characteristics we examined were demographic factors, drinking history, severity of alcohol withdrawal, and liver function

Patients and Methods

During the study period October 20, 1979 through March 1, 1980, 621 patients were available for study purposes. Patients were admitted to the Beth Israel Medical Center Alcoholism Treatment Program inpatient unit, a voluntary, 50-bed service which uses a tapering dose of phenobarbital to withdraw patients from physical dependence on alcohol. The criteria for admission to the Alcoholism Unit are the PRSO criteria for alcohol-withdrawal syndrome, consisting of elevated blood pressure, tachycardia, fever, or in-

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TABLE 1

Classification by Opiate and Nonopiate Drug Use of 621 Alcoholic Patients

Drug use classification	n (%)
I: Opiates and other drugs	330 (53.1)
II: Other drugs only	60 (9.7)
III: No drugs	231 (37.2)
TOTAL	621 (100.0)

ability to stop drinking despite medical supervision.

All of the patients had been drinking for many years and consumed significant amounts of alcohol on a sustained basis. All patients voluntarily sought treatment. Some were referred by social agencies, but the patterns of referral were similar for the different groups. All information was collected and charted by alcoholism counselors and nurses. Data were obtained from patients' charts by trained abstractors. Patients were categorized as to drug use on the basis of the history obtained by the counselor and nurse and through the use of urine toxicology. If either the patient's history or urine findings was positive, the patient was assigned to the drug-use category.

Results

The distribution of drug use among patients (Table 1) shows that more than half (53.1%) used opiates and other drugs (group I).

TABLE 2

Age Distribution, 621 Alcoholic Patients, by Drug Use

	Use of Drugs		
	Opiates	Other drugs only	No drugs
Age	n (%)	n (%)	n (%)
≤24	5 (2)	5 (8)	6 (3)
25–34	122 (36)	14 (24)	29 (12)
35–44	125 (38)	16 (26)	87 (38)
45–54	68 (21)	15 (25)	66 (30)
55–64	9 (3)	10 (17)	29 (13)
≥65	1	0 (0)	10 (4)
TOTAL	300 (100)	60 (100)	231 (100)
mean	38.1	41.8	44.9
median	37.1	41.2	43.3

Oneway analysis of variance: $F_{(2,618)} = 33.91; p = 0.0000$. Mean ages of groups differ significantly. By Scheffé procedure, opiate users differ from users of other drugs and from nondrug users (at 0.10 level).

Of the 330 patients in this group, 193 (58.5%) had used heroin in the past, 122 (37.0%) were maintained on methadone, and 15 (4.5%) were using either heroin or street methadone. All 122 patients who were being treated with methadone were continued in this treatment during their hospital stay. None of the patients in the study required treatment for opiate withdrawal.

Only a small percentage of patients (9.7%) used other drugs but not opiates (group II); 37.2% used alcohol alone (group III). Opiate users were

TABLE 3

Sex and Ethnic Distribution, 621 Alcoholic Patients, by Drug Use

	Use of Drugs		
	Opiates	Other drugs only	No drugs
	n (%)	n (%)	n (%)
Sex			
Male	267 (81)	50 (83)	210 (91)
Female	63 (19)	10 (17)	21 (9)
TOTAL	330 (100)	60 (100)	231 (100)
	χ^2 (2df) = 10.699	$p = .0048$	
Ethnicity			
White	44 (16)	30 (53)	91 (44)
Black	163 (57)	17 (30)	72 (35)
Hispanic	76 (27)	10 (17)	41 (20)
Other	1	0	3 (1)
TOTAL	284 (100)	57 (100)	207 (100)
Unknown	6	3	24
	χ^2 (6df) = 65.68	$p = .0000$	

TABLE 4

Drinking History, 621 Alcoholic Patients, by Drug Use

	Use of Drugs		
	Opiates	Other drugs only	No drugs
Drinking Years	n (%)	n (%)	n (%)
≤5	125 (38)	10 (17)	28 (12)
6–10	89 (27)	18 (31)	43 (19)
11–15	54 (16)	3 (5)	46 (21)
16–20	32 (10)	19 (32)	52 (23)
≤21	29 (9)	9 (15)	55 (25)
TOTAL	329 (100)	59 (100)	224 (100)
Unknown	1	1	7
mean	9.66	14.56	16.27
median	8.00	12.00	15.27
	χ^2 (8df) = 91.13	$p = 0.0000$	

Oneway analysis of variance: $F_{(2,609)} + 44.39; p = 0.0000$. Mean years drinking of groups differ significantly. By Scheffé procedure, opiate users differ from the other two groups (0.10 level). Age was controlled by analysis of covariance: $F_{(2,551)} = 25.9; p < 0.001$.

TABLE 5

Duration of Phenobarbital Treatment, 621 Alcoholic Patients, by Drug Use

	Use of Drugs		
	Opiates	Other drugs only	No drugs
Duration days	n (%)	n (%)	n (%)
0	31 (10)	1 (2)	18 (8)
1	109 (33)	13 (22)	55 (24)
2	11 (3)	3 (6)	8 (4)
3	2 (7)	5 (8)	14 (6)
4	135 (42)	32 (54)	120 (53)
⩾5	17 (5)	5 (8)	12 (5)
TOTAL	325 (100)	59 (100)	227 (100)
Unknown	5	1	4
mean	2.55	3.25	2.91
median	3.02	3.73	3.65
	χ^2 (10df) = 15.02	p = 0.13	

Oneway analysis of variance: $F_{(2,608)} = 6.25$; $p = 0.002$. Mean duration of phenobarbital treatment of groups differs significantly. By Scheffé procedure, opiate users differ from other two groups.

significantly younger than other subjects, users of other drugs being of intermediate age (Table 2).

The sex and ethnic distributions of the groups are significantly different. A larger proportion of opiate users than of the other groups were female and black. Among blacks and hispanics, sex distributions did not differ significantly; among whites they did. This suggests that the larger proportion of females among opiate users is not entirely explained by the ethnic group distributions of the drug-use groups. Among males, when ethnicity was controlled, whites and hispanics continued to show significantly different age distributions, while blacks did not. The number of females in the study was not sufficient for a similar exploration. The results in males, however, indicate that the difference in age distribution of the groups is not explained by differences in sex and ethnicity (Table 3).

Opiate users had shorter drinking histories (Table 4) than other subjects, with a mean of 9.66 years (means in the other two groups were 14.56 and 16.27). When age is controlled by analysis of covariance, the groups continue to differ significantly in drinking histories.

The duration of phenobarbital treatment among opiate users differed significantly from the other two groups (Table 5). The opiate users required fewer days of phenobarbital treatment; their mean was 2.6 days, compared to 3.3 and 2.9 for the other two groups.

The groups differed in liver function test results. Opiate users included a higher proportion of subjects with abnormal SGOT, SGPT, alkaline phosphatase, and albumin globulin ratio than the other groups (Table 6).

Discussion

The major finding of this study is that alcoholics who use or have used opiates are different from alcoholics who have never used opiates. Alcoholics using nonopiate drugs appeared to resemble alcoholics who did not use drugs other than alcohol, rather than opiate-using alcoholics. Demographic data from this study indicate that the alcohol-opiate group of whites and hispanics is younger than the alcohol alone group. The younger age of opiate alcoholics has been reported by a number of previous investigators. However, these investigators did not separate opiate users using alcohol from those who used other drugs. Why blacks did not fit into this pattern is unclear. The alcohol-opiate group contained more blacks and females than the alcohol-alone group, a finding which may be related to the fact that the patient population in our study comes primarily from the lowest social and economic sector.

Number of years spent drinking and days of treatment with phenobarbital is less in the opiate group. Therefore, the alcohol-opiate population appears to be newer to alcohol abuse, and the severity of their withdrawal from alcohol is not as great as the other group. This finding may be consistent with reports (7) that opiate-using alcoholics consume less alcohol than "pure" alcoholics.

Ashley (6) et al found more cirrhosis in "pure" alcoholics than in a drug-abusing group of alcoholics, whereas our study found a higher incidence of abnormal liver function tests in the opiate-using alcoholics. Our finding is perhaps indicative of an additive effect of liver damage resulting from viral hepatitis and the further complication of alcohol toxicity (8).

The age difference among the groups in our study and in others may be related to the higher incidence of liver and other diseases in opiate-using alcoholics, which might lead to a shorter life expectancy among the opiate-using group.

The liver disease seen in the opiate-alcohol group may alter their ability to metabolize phenobarbital. Alvin (9) has shown that in patients with cirrhosis, phenobarbital has a significantly

TABLE 6
Liver Function Tests

Test	Opiates n (%)	Other drugs only n (%)	No drugs n (%)	Totals n (%)
SGOT				
Normal	45 (14)	27 (46)	91 (40)	163 (27)
Abnormal	276 (86)	32 (54)	135 (60)	443 (73)
Total known	321 (100)	59 (100)	226 (100)	606 (100)
	χ^2 (2df) = 58.16	$p < 0.001$		
SGPT				
Normal	135 (42)	38 (64)	145 (64)	318 (52)
Abnormal	186 (58)	21 (36)	81 (36)	288 (48)
Total known	321 (100)	59 (100)	226 (100)	606 (100)
	χ^2 (2df) = 29.63	$p < 0.001$		
Alkaline phosphate				
Normal	168 (52)	42 (71)	148 (66)	358 (59)
Abnormal	153 (48)	17 (29)	77 (34)	247 (41)
Total known	321 (100)	59 (100)	225 (100)	605 (100)
	χ^2 (2df) = 13.86	$p < 0.001$		
Albumin globulin ratio				
Normal	147 (47)	51 (87)	179 (82)	377 (63)
Abnormal	169 (53)	8 (14)	40 (18)	217 (37)
Total known	316 (100)	59 (100)	219 (100)	594 (100)
	χ^2 (2df) = 84.22	$p < 0.001$		

greater half-life than in normal controls, and that this correlates with the presence of low serum albumin. Therefore, persons in the opiate-alcohol group who have low levels of serum albumin may not need as much phenobarbital when they do go into withdrawal. Another possibility is that the opiate-alcohol group required less phenobarbital because 37% were maintained on methadone, thereby decreasing their need for phenobarbital.

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Orthotopic, Segmental Small-Bowel Transplantation in the Rat

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Abstract

A technique for orthotopic, segmental small-bowel transplantation in the rat is described. A 10-cm segment of small bowel with its vascular pedicle is harvested from a donor rat. The superior mesenteric artery is anastomosed to the aorta of the recipient in an end-to-side fashion. The superior mesenteric vein is anastomosed in a similar fashion to the inferior vena cava 6 mm distal to the arterial anastomosis. Total ischemia time has always been less than 1 hr. A similar length of bowel is then resected in the recipient. The graft is anastomosed, in continuity, with the recipient's bowel, utilizing end-to-end anastomoses performed with continuous 7-0 silk. Isografts were transplanted in ten consecutive rats. Parameters studied included body weight, quality of stool, microscopic anatomy, and recipient survival. This group showed universal survival, significant increases in weight, normal histology of the graft and/or adjacent bowel, and no evidence of loss of function. This model will be useful to study of the immunobiology of small-bowel allografts.

A large number of patients suffering from acute mesenteric vascular occlusion or inflammatory bowel disease require extensive small-bowel resection. Despite the advent of intravenous hyperalimentation, many patients continue to suffer diarrhea, weight loss, and nutritional deficiencies. These factors, combined with the prohibitive cost of both supplies and daily management, make small-bowel transplantation a valid alternative. Attempts at small-bowel transplantation with conventional immunosuppression have been uniformly unsuccessful (1–4). The availability of Cyclosporin A has, however, changed the perspective in transplantation, and organ grafting has assumed a new role in the management of many diseases. In order to investigate the immunobiology of intestinal allografts, we deemed it essential to develop a reliable experimental model in small laboratory animals.

In this report we describe in detail a technique for orthotopic, segmental bowel transplantation in the rat which may also have clinical bearings. Recipients of isografts showed universal survival with no pathologic findings. This model is currently being used in our laboratory to study the effect of various immunosuppressive regimens on the survival of intestinal allografts.

Materials and Methods

Animals. Inbred, Lewis rats weighing 300–400 gm were used as both donors and recipients. They were obtained from a commercial source (Harlan-Sprague Dawley, Inc., Walkersville, MD.)

Preoperative Care. Both donor and recipient animals were allowed food and water ad lib. No bowel preparations or antibiotics were administered.

Harvesting of Donor Small Bowel. The donor rat was anesthetized with Nembutal (30 mg/kg in-

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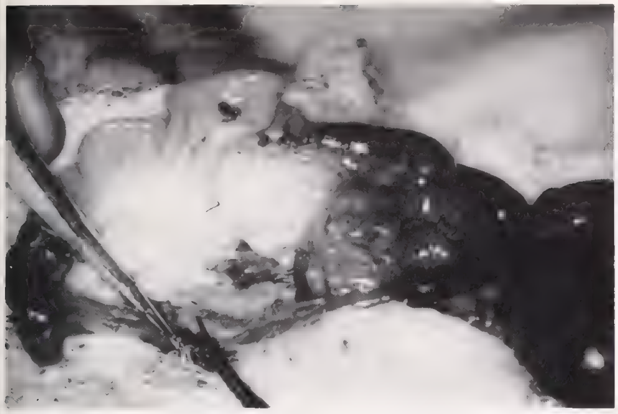

Fig. 1. Segment of donor jejunum showing discoloration after perfusion.

traperitoneally), supplemented with ether. After the abdominal wall was shaved and cleaned with alcohol, the animal was secured on its back on a corkboard. The operation was performed under a microscope using 10× magnification. The abdomen was opened in the midline from xiphoid to pubis. The bowel was eviscerated and a 10-cm segment of jejunum selected. The blood supply of this segment was carefully isolated at its origin. The retroperitoneum was opened and the aorta exposed at the origin of the superior mesenteric artery (SMA). Those branches not contributing directly to the selected intestinal segment were ligated with 7-0 silk. Similarly, all tributaries to the superior mesenteric vein (SMV) were exposed with surrounding noncontributory veins ligated. After all the vascular attachments of the small intestinal segment had been isolated, a 7-0 silk ligature was tied around the aorta just proximal to the SMA. The SMA was then perfused with 3 ml of normal saline containing 0.1 ml of heparin and 0.4 ml of 1% lidocaine. This caused a discoloration of the desired small-bowel segment (Fig. 1). It also served as a clear demarcation of the extent of the isolated blood supply. The SMV was then divided at the origin of the segmental tributary. At this point, the bowel segment was divided and removed from the remaining small bowel. The graft thus consisted of a 10-cm segment of jejunum along with its vascular supply. Intraluminal contents were removed by both compression and irrigation with saline.

Transplantation of Small-Bowel Isografts. The recipient rat was similarly anesthetized, and its abdominal wall was shaved and cleansed with alcohol. The abdominal cavity was entered through a midline incision. A segment of abdominal aorta and inferior vena cava (IVC) below the renal

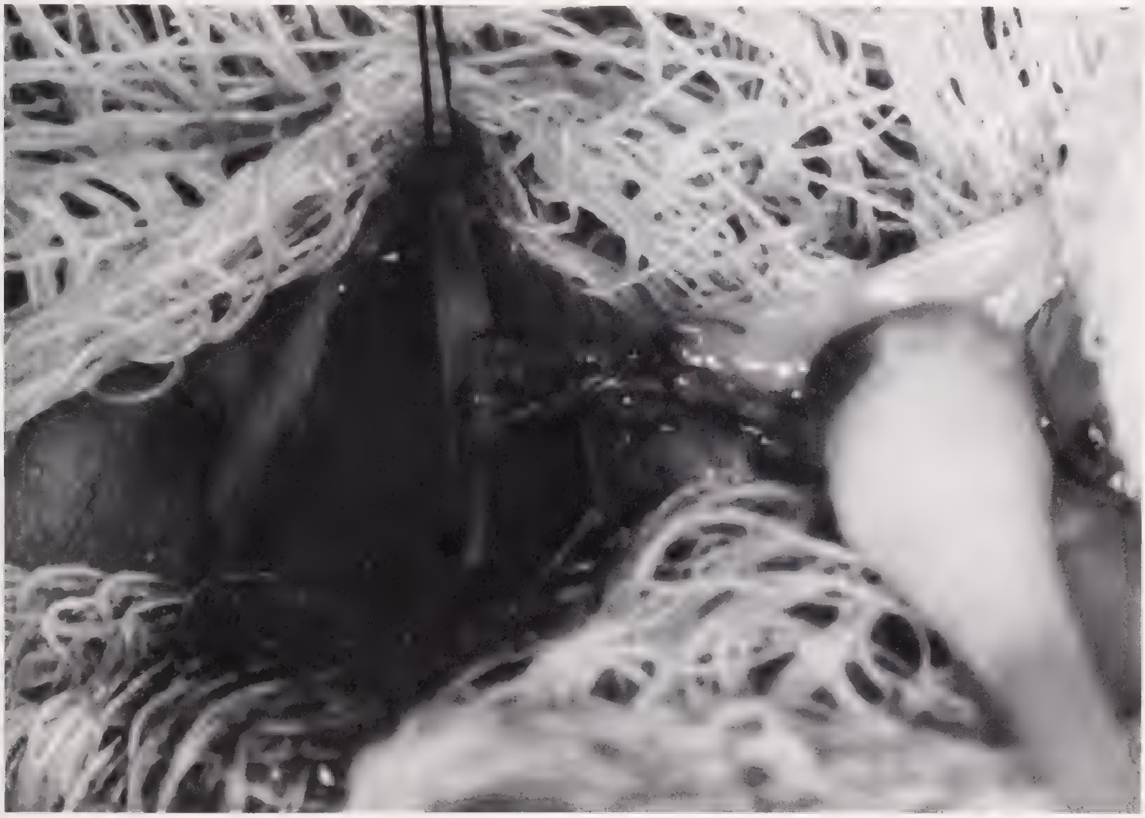

Fig. 2. View of completed vascular anastomosis of superior mesenteric artery to recipient aorta and superior mesenteric vein to recipient inferior vena cava.

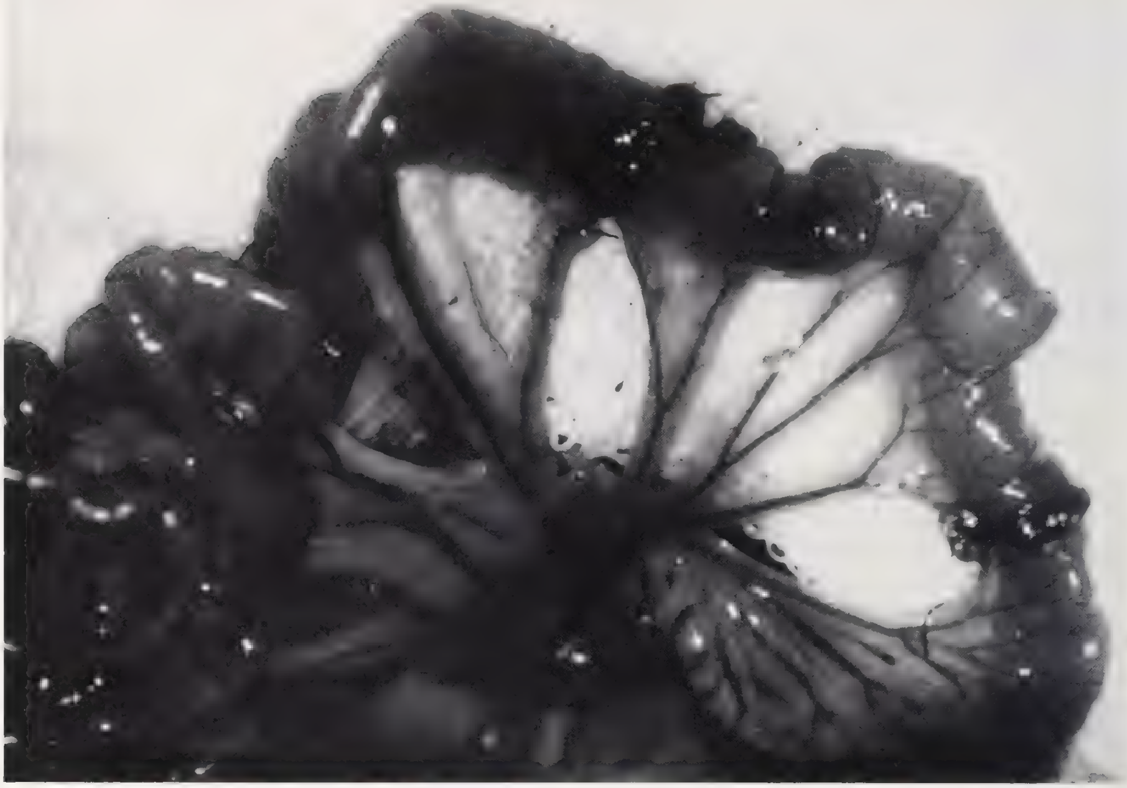

Fig. 3. View of graft anastomosed in continuity with recipient bowel.

vessels was dissected and freed from peritoneum and connective tissue. Two proximal and distal loops of 4-0 silk were placed around the aorta and IVC below the renal vessels to occlude circulation temporarily during vascular anastomosis. Revascularization was accomplished by initially anastomosing the superior mesenteric vein to the recipient IVC in an end-to-side fashion and then anastomosing the superior mesenteric artery to the recipient infrarenal aorta in an end-to-side fashion 5 mm proximal to the venous anastomosis (Fig. 2). The anastomoses were done using 10-0 nylon in a continuous fashion. Upon completion of the anastomoses, the occluding loops were removed and circulation reestablished. Hemostasis from needle holes was controlled by gentle pressure with cotton swabs and with oxidized regenerated cellulose (Surgicel). The revascularized intestinal segment turned pink immediately. Total ischemia time was kept below 1 hr.

Intestinal Anastomosis. In the recipient rat, a small-bowel resection of a length of bowel similar to the graft was performed prior to bowel anastomosis. This was done to ensure that the total length of small bowel in the recipient would be the same after transplantation. Both the proximal and the distal ends of the graft were anastomosed, in continuity, to the recipient small bowel. This was done as a one-layered enteroenterostomy using 7-0 silk in a continuous fashion (Fig. 3). The graft was then placed in the abdominal cavity without hindrance to its blood supply. The peritoneum and muscle layers were closed with a continuous suture of 4-0 silk.

Postoperative Care. Postoperatively, the recipient was placed back into its cage and allowed to resume a normal diet.

Survival, weight, and quality of stool were noted daily. No antibiotic therapy was administered at any time. At selected intervals, full-thickness wedge biopsies of grafted and native bowel were taken and preserved in formalin.

Results

The rats receiving isografts in this series all survived longer than 70 days; the longest survival time was over 130 days at the time of writing. All animals demonstrated similar changes in weight both perioperatively and postoperatively (Fig. 4). In the first postoperative days, all rats showed an average loss of body weight of 9%. The average weight on the day of surgery was 344 ± 33 gm; on day 5 postoperatively, it was 314 ± 30 gm. However, at that point all rats exhibited gains in weight, and by

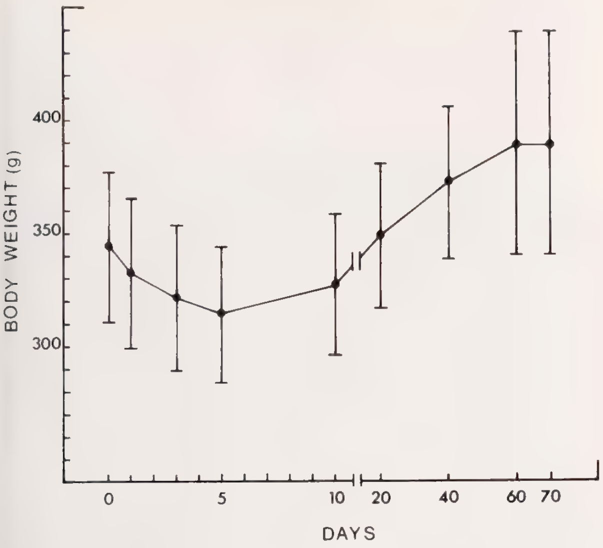

Fig. 4. Average change in weights of ten consecutive rats after receiving small-bowel isografts.

day 25 all surpassed their initial weight. Stabilization of weight occurred between day 60 (388 gm ± 58) and day 70 (390 gm ± 50). None of the rats developed diarrhea or bowel obstruction.

Gross examination of four isografts postoperatively revealed numerous serosal adhesions and patent vascular anastomoses without evidence of dilatation or obstruction.

Microscopic examination of three intestinal grafts from three recipients taken 60, 100, and 120 days after surgery showed intact mucosa. The population of epithelial cells retained normal proportion and configuration. However, mild thickening of muscularis mucosae and muscular layers was noted (Fig. 5).

Discussion

Microvascular surgical technique has allowed the use of small animals as experimental models in studying the behavior of vascularized organ allografts. The rat, in particular, offers many advantages as an animal model (5, 6). The availability of inbred strains with well-defined major histocompatibility antigens allows the study of the various factors that influence graft survival while the genetic barrier is kept constant. Furthermore, the rat offers economic advantages with respect to animal cost, maintenance, and use of operating room space when compared with other animals. Finally, rats exhibit a high natural resistance to infection, which makes it possible to perform surgery with clean, nonsterile instruments, greatly simplifying the experimental procedures.

Other investigators have devised experimental techniques and models to study small-bowel transplantation. Lillehei and associates reported survival of more than seven years with functional canine small-bowel autografts (7); allografts were rejected between the sixth and ninth postoperative day (8). Mack et al (9) and Barnett et al (10) carried out segmental small-bowel transplantation in dogs bringing out the distal end as a stoma which could be observed for changes caused by rejection. Preston et al (1) transplanted intestinal allografts in the necks of dogs by anastomosing vessels of the graft to the carotid artery and jugular veins of the recipient. However, a large proportion of the grafts were labeled as surgical failures. Monchik and Russell (4) described a model of auxiliary, heterotopic small-bowel transplants in the rat with venous outflow into the caval vein and with the duodenum and ileum brought to the abdominal wall as duodenostomy and ileostomy. They reported a 20% mortality. Kort et al (11) described a method of orthotopic, total small-bowel transplantation

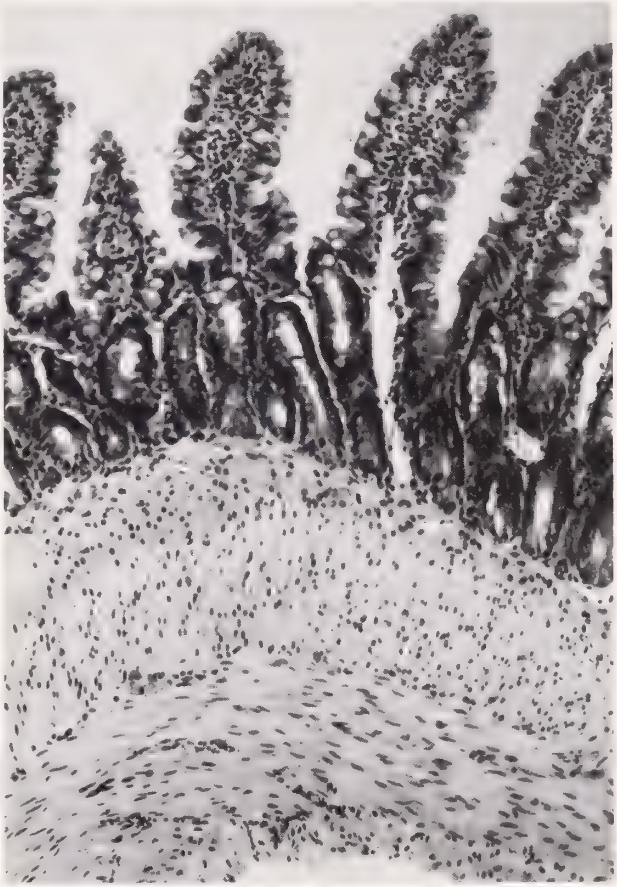

Fig. 5. Photomicrograph of jejunum isograft biopsied 100 days after transplantation (hematoxylin and eosin, original magnification 250×).

in the rat. The mesenteric vein was anastomosed to the portal vein. Nearly one-third of the recipients, however, died within 48 hr, primarily owing to portal vein thrombosis.

Advantages of This Technique. The technique of small-bowel transplantation described here offers several major advantages over published techniques. First, the graft is transplanted in its anatomic position and the technique itself has direct clinical bearings. Furthermore, the viability of the host in the present model is dependent on the functional integrity of the graft, as it would be in clinical situations. Thus, observations regarding the immunobiology of small-bowel allografts in this model have greater clinical significance than studies performed with heterotopic or auxiliary models. In addition, since a portion of the recipient small intestine remains intact, histologic comparison of grafted and native tissue can be easily done.

From a surgical standpoint, the most critical aspect of the technique is the vascular anastomoses, since the caliber of the vessels in question is usually less than 1 mm. During the early phase of development of this technique, we noted that imperfect vascular anastomoses led to vascular occlusion with subsequent ischemic bowel, peritonitis, and death of the recipient within 36 hr. Once the technique is mastered, however, the procedure can be carried out with an average operating time of 2½ hr.

The excellent survival, maintenance of nutritional status, and lack of serious complications with isografts make this technique a useful model in transplant research.

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Large-Bowel Dilatation Due to Nonincarcerated Hernias

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Abstract

The increasing rate of colonic carcinomas makes large-bowel obstruction a common problem that gastroenterologists and surgeons have to deal with. Sometimes, however, we are faced with colonic obstruction of unknown or uncertain origin. Four cases are presented here, one of severe constipation and three with acute colonic distention, in whom the cause is presumed to be nonincarcerated inguinal hernias of the sigmoid colon. Three of them were left-sided sliding hernias and one was a right-sided indirect hernia. All patients were successfully treated by simple hernia repair under local anesthesia. No reports on nonincarcerated inguinal hernias as the cause of large-bowel dilatation and obstruction were found in an extensive review of the medical literature.

Case Reports

Case 1: An 82-year-old white man underwent routine barium-enema examination for constipation of two months' duration. X-rays showed dilatation of the large bowel with sigmoid colon in the left inguinal region (Fig. 1). On physical examination, a left inguinal hernia was found, with no sign of incarceration.

A hernioplasty was performed with the patient under local anesthesia. A sliding hernia of the sigmoid colon was found. After surgery, the patient had completely normal bowel movements; at six months follow-up, a plain abdominal X-ray was normal, as were the patient's bowel habits.

Case 2: A 65-year-old white patient was admitted to the emergency ward with abdominal pain of three days' duration. Plain abdominal X-ray showed a markedly distended large bowel and some distention of the small bowel. Barium enema demonstrated that the sigmoid colon was passing through a left inguinal hernia, with no obstruction (Fig. 2).

Under direct TV–X-ray screener, manual pressure on the left inguinal region showed this to be a sliding hernia of the sigmoid colon, with no signs of incarceration. After hernioplasty with the patient under local anesthesia, the patient did well. No further complaints were voiced at six months' follow-up. Repeat plain abdominal X-rays showed no bowel distention or obstruction.

Case 3: An 81-year-old white patient was admitted to the emergency ward for bouts of abdominal distention and pain of four months' duration. Plain abdominal X-ray showed large- and small-bowel distention. Barium enema showed the sigmoid to be within a left inguinal hernia. No lumen obstruction was found (Fig. 3). A simple local-anesthesia hernioplasty was performed; recovery was uneventful. The patient was doing very well at 6-month follow-up.

Case 4: An 80-year-old white man was admitted to the emergency ward for abdominal pain and distention, recurrent for three years. In a complete checkup at another hospital, the patient had been found to have a huge diaphragmatic hernia with herniation of the stomach into the chest; this was presumed to be the cause of his recurrent pain.

Plain abdominal and chest X-rays showed the stomach to be in the chest and also revealed distention of the large and small bowels (Fig. 4). On barium-enema examination, the sigmoid

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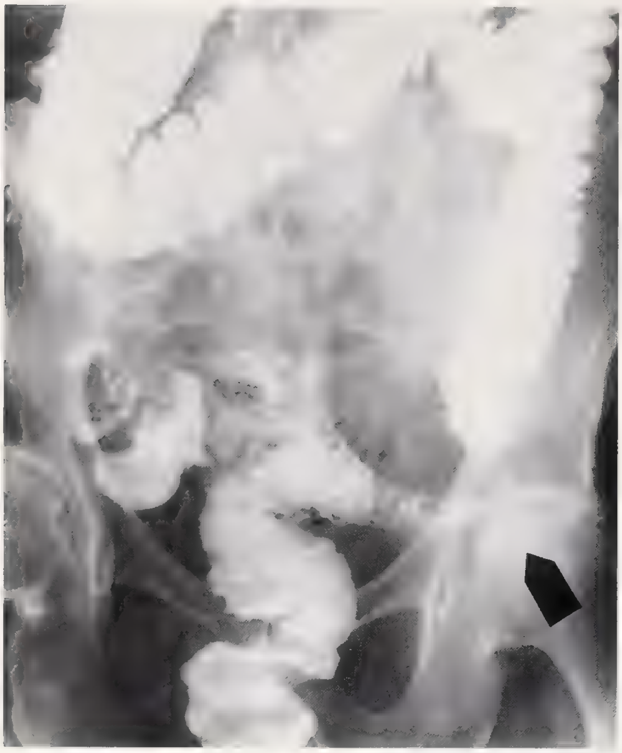

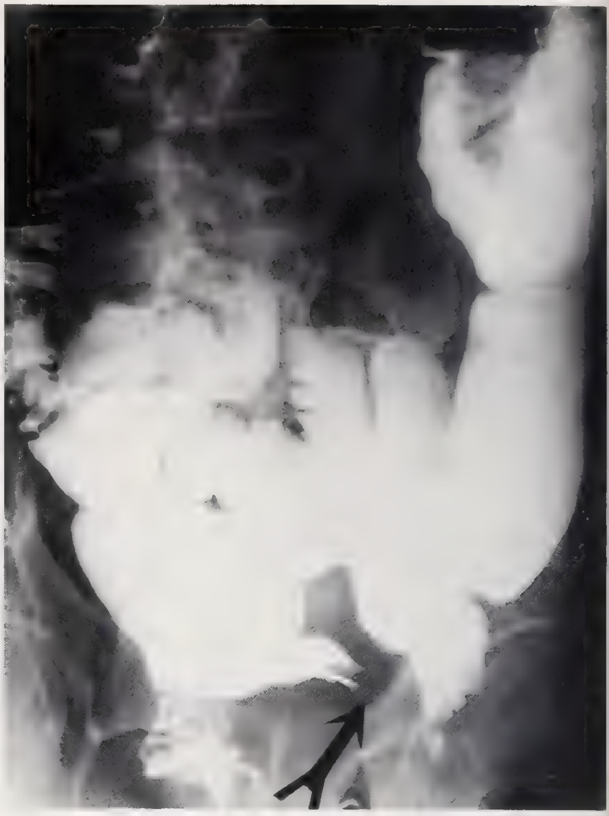

Fig. 1. Left Case 1. Barium enema showing passage through sigmoid colon herniated into left inguinal canal.
Fig. 2. Right Case 2. Barium enema with cutoff in area of hernial sac.

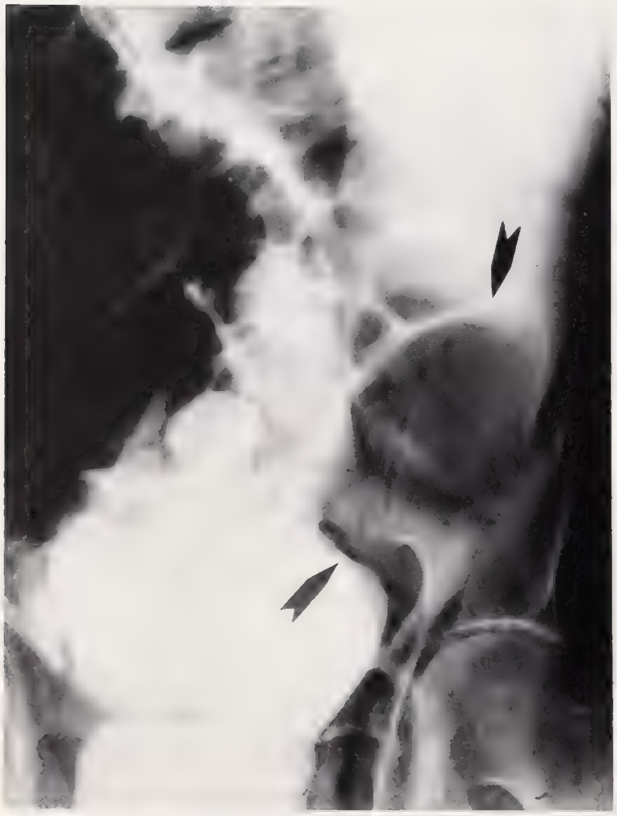

Fig. 3. Case 3. Early in enema showing extended dilatation of bowel.

colon was found in a right inguinal hernia, with no lumen obstruction. The hernia was repaired with the patient under local anesthesia, and all signs of colonic distention disappeared by the second day. Two-month follow-up showed no recurrent abdominal pain or bowel distention.

Discussion

According to Morton et al (1), the discovery of a greatly distended large bowel in the absence of significant small-bowel involvement is almost pathognomonic of obstruction or mesenteric vascular involvement. The usual causes of large-bowel obstruction are carcinoma, diverticulitis, and volvulus (2); the patients are usually elderly (3). Uncommonly, cases of clinical and radiologic large-bowel obstruction are found in which no mechanical or vascular factor can be found. This condition has been given a great number of designations, including idiopathic pseudoobstruction (4); intestinal pseudoobstruction (5); ileus of the colon (1); spastic ileus (6); and pseudoobstruction of the colon (7). Truly idiopathic large-bowel distention is rare. A large number of diseases and factors, such as impaired renal function, left ventricular failure, and electrolytic disturbances,

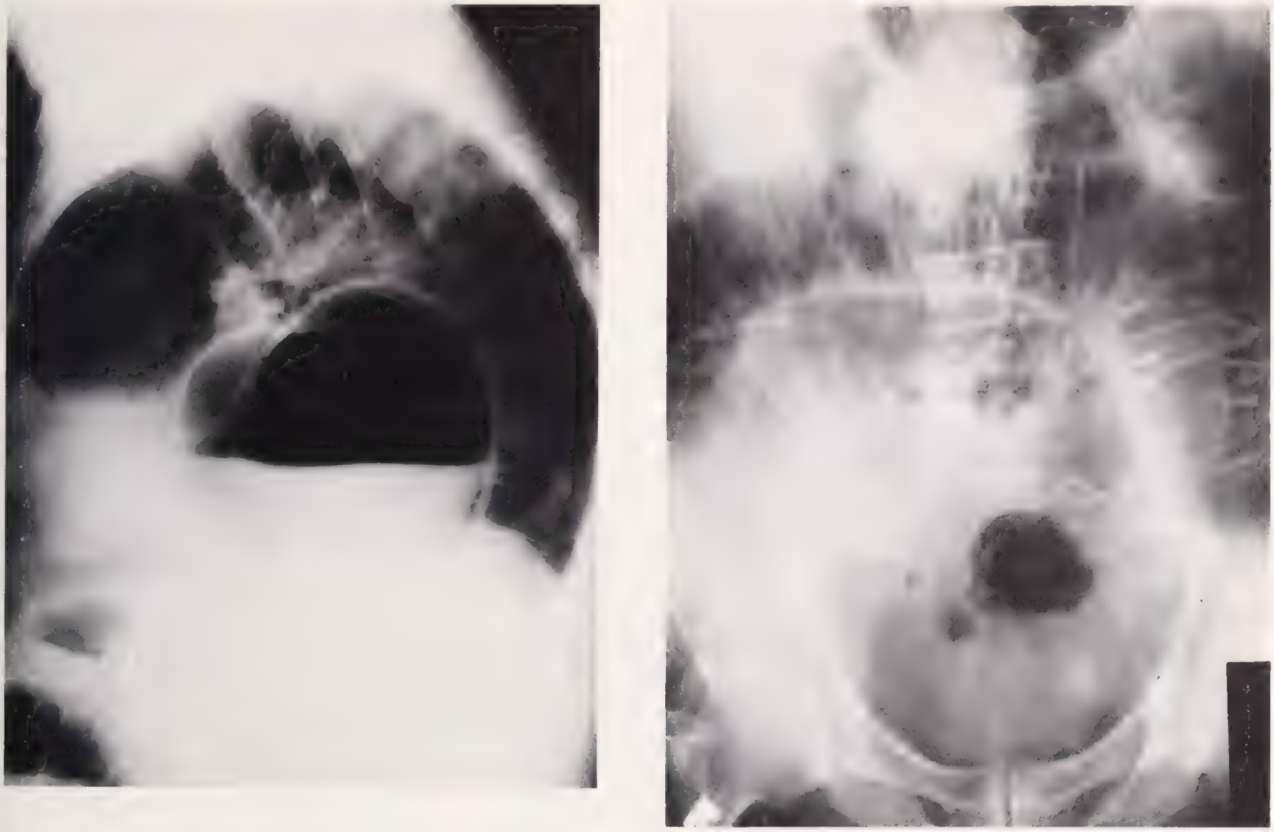

Fig. 4. **Left** Case 4. Lateral chest X-ray showing huge diaphragmatic hernia. **Right** Same patient; plain abdominal X-ray showing dilatation of large bowel and part of small bowel.

can lead to colonic distention. Other causes are lead poisoning, porphyria, morphine overdosage, abdominal inflammatory conditions, lower abdominal operations, septicemia, and acute dysentery. Less well-known causes include ganglionic block agents, myxedema, psychosis, antiparkinsonian drugs (8), Ogilvie's syndrome (9), blunt trauma (10), Chagas' disease, scleroderma, and amyloidosis (3).

According to Norton et al (11), colonic distention is four times more common in men than in women. Cases diagnosed before laparotomy can usually be treated conservatively with good response and resolution of the distention within a few days. However, the absence of an apparent cause can lead to delayed or precipitous treatment. Surgical treatment generally involves the opening of a stoma (particularly cecostomy), but despite the fact that this is a minor surgical procedure, the mortality rate is high (32%) (2). Conservative treatment includes nasogastric intubation, correction of fluid and electrolytic imbalance and, if possible, decompression of the colon with a flatus tube (3, 6, 10). Lack of response to this treatment accompanied by increasing cecal size may suggest that resort to surgery is the only way to resolve the distention.

In Bachulis and Smith's series (10), two patients' colons were decompressed with a colonoscope. Colonoscopy, however, requires introduction of air to identify the colonic lumen and facilitate passage of the tubes. Introducing this new amount of air into a distended colon could precipitate cecal disruption; this hazard has to be taken into account when using the diagnostic procedure for colonic distention.

Reports of four patients are presented here. One had chronic constipation and three had acute colonic distention. No cause was found in any of the four, aside from inguinal hernias of the sigmoid colon (of the sliding type in three left-sided hernias and an indirect right-sided hernia in one case). No clinical or radiologic evidence of true obstruction was demonstrated. With repair of the hernias, these colonic dilatations and the constipation disappeared, and the patients did well afterward.

Despite passage of the sigmoid colon in the hernial sac, barium passed freely through it. In all cases, a flatus tube was introduced, and it permitted the passage of flatus and liquid feces when concomitant manual pressure was exerted on the hernia.

In our extensive search of the literature, far beyond the references cited here, we did not find any reference to a case in which a nonincarcerated hernia of the sigmoid colon could have produced a large-bowel dilatation or constipation. Two collective reviews citing more than 300 references also failed to describe this entity (9, 12).

We believe that inguinal hernias of the sigmoid colon, particularly of the sliding type, even without incarceration, have to be added to the differential diagnosis when dealing with dilatation of the colon when no other cause is found.

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A New Look at Acute Cholecystitis

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Abstract

Acute cholecystitis remains one of the most frequent abdominal emergencies, yet many aspects of its management remain controversial. This prospective study of 366 consecutive operations in 1970–1982 for acute cholecystitis was conceived to assess the value of early surgery and routine operative cholangiography in the improvement of both immediate and long-term results. The patients ranged from 26 to 82 years of age; about half were over 60 years. Gangrenous changes were encountered in 20.2%, and perforated cholecystitis in 3.6%; 6.8% had no biliary calculi. Cholecystectomy was performed in all patients. A technically successful operative fluorocholangiography via the cystic duct was performed in 98% of patients; no complications due to this procedure were encountered, and was 94% accurate. Bacteriologic data disclosed a predominance of *Escherichia coli* and other gram-negative bacilli. The aminoglycosides and newer cephaloridines were the most effective antibiotics. There were 4 postoperative deaths (1.1%) and 1.3% residual stones. We conclude that a therapeutic policy combining early and definitive surgery with routine operative fluorocholangiography improves results and decreases the incidence of residual ductal stones.

Surgery for acute cholecystitis has replaced appendicitis as the most serious and most frequent intra abdominal emergency. Many advances in the diagnostic and therapeutic field and especially sonography have contributed to the treatment of acute cholecystitis. Despite this, several aspects of its management remain controversial (1–3).

The principal areas of controversy include the following:

Timing of surgery. In many centers, the advantages of early surgery (4, 5) have been emphasized; in others, the conservative approach (6, 7) with elective surgery at a later date has been preferred. Comparative studies attempting a statistically valid comparison between the 2 concepts have given equivocal results (8, 9).

Routine use of operative cholangiography. Operative cholangiography in elective cases has been performed in many surgical centers and has decreased mortality and morbidity. However, in acute cases, its use is infrequent, probably for technical reasons. Few, if any, authors recommend its routine use.

Extent of surgery. While cholecystostomy historically has had priority over cholecystectomy, its use in elective operations has practically ceased. In acute cases, however, many surgeons still recommend this limited procedure in a wide spectrum of clinical circumstances.

This prospective study of 366 consecutive operations for acute cholecystitis was conceived to assess the value of a therapeutic policy consisting of the following principles:

1. Early surgery, performed within 48 hours after full diagnostic investigation and adequate preparation.
2. Routine operative fluorocholangiography to avoid both unnecessary explorations and residual pathological conditions in the common bile duct.
3. One-stage definitive procedure, including cholecystectomy and elimination of stones or fibrosis in the common bile duct.

Temporary measures, such as cholecystectomy, were not used in this series.

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TABLE 1
Clinical Characteristics of 366 Patients with Acute Cholecystitis, 1970–1982

	No. of patients	%
Sex		
Male	146	39.9
Female	220	60.1
Age		
Below 30	9	2.4
30–49	72	19.6
50–59	98	26.8
60–69	113	30.8
70–79	56	15.3
80–89	17	4.6
90 & above	1	0.3

TABLE 2
Duration of Biliary Symptoms, 366 Patients with Acute Cholecystitis

	No. of patients	%
Less than 1 month	201	55.7
1 month–12 months	75	20.5
More than 12 months	90	29.8

TABLE 3
Jaundice at Time of Surgery, 366 Patients with Acute Cholecystitis

	No. of patients	%
None (0–0.9 mg)	280	76.5
Moderate (1.0–2.9 mg)	32	8.7
Severe (3.0–30.0 mg)	54	14.8

TABLE 4
Choledochal Diameter, 366 Patients with Acute Cholecystitis

	Unknown	2–6 mm	7.9 mm	10.12 mm	13–40 mm
Number	18	111	108	65	59
%	4.9	31.7	29.5	17.8	16.1
% Incidence of C.B.D. stones	1	7	24	69	
		p	0.001		

TABLE 5
Number of Stones in Gall Bladder, 366 Patients with Acute Cholecystitis

	0	1–10	Above 10
Number	25	154	187
%	6.8	42.1	51.1

TABLE 6
Histologic Findings, 366 Patients with Acute Cholecystitis

	Cases	%
Acute suppurative	279	76.2
Gangrenous	74	20.2
Perforation	13	3.6

An additional aim of this study was a review of bile bacteriology focusing on sensitivity to the newer antibiotics introduced over the last decade.

Patients, Materials, and Methods

This group of 366 patients comprises all patients with acute cholecystitis operated on during the 13-year period 1970–1982 at the Department of Surgery headed by the senior author. Only histologically confirmed cases were included; patients showing malignant changes were excluded. During the period under study, 1,438 elective biliary procedures were performed on patients with chronic cholecystis and are used here to compare certain features with the acute cases reported. Ultrasonography has virtually replaced other diagnostic techniques and was the only method used in most acute cases in this study.

Routine operative fluorocholangiography using an image intensifer and TV screen (Sire Mobil II) was the absolute rule in this series and was omitted in 6 cases only.

Bile cultures and sensitivity studies were performed in all patients.

The accumulated data on both acute and elective cases were recorded on data sheets including 56 relevant items on each case and analyzed using an MPT 100 Data General Computer. Chi-square determination was used for statistical analysis; all values of p smaller than 0.05 were considered significant.

Results

Almost 50% of the patients were above 60; 40% were males (Table 1). In more than 50% of the patients, the acute attack was the first manifestation of biliary-tract disease (Table 2). Jaundice was present in almost 25% of the patients (Table 3) and about 30% of them had some choledocal enlargement (Table 4).

The presence of hyperbilirubinemia (bilirubin above 3 mg%) and choledocal size above 13 mm correlated well with presence of CBD stones (Table 5). Patients with lower, borderline increases over normal values had relatively low rates of common bile duct (CBD) stones.

Acalculous cholecystitis was present in 6.8% of this group and either gangrenous changes or perforations were observed in almost 24% of the patients (Table 6). Diabetes mellitus was noted in 16% of the patients, almost twice the rate observed in elective biliary surgery in our hospital. The diabetic patients displayed high rates of septic bile (80%) and high rates of septic complications; however, their mortality was not significantly higher than in nondiabetics. All patients in this series had their gallbladder removed; no cholecystostomy was performed.

The rate of choledocal exploration was almost 30%, only 6% being negative (Table 7). About 6% of the patients underwent choledocoduodenostomy. Septic bile was present in gallbladder in 57.6%, *Escherichia coli* and *Klebsiella* being by far the most common isolates (Table 8). The study of sensitivity to the newer antibiotics has shown the antibiotics of choice to be the aminoglycosides as well as second and third generation cephaloridines (Table 9).

The incidence of diagnosed residual stones in this series was 1.3%, and there were no cases of postoperative stricture or traumatic injury to the choledocus (Table 10).

There were 4 deaths in this group (1.1%), the mortality correlating significantly and positively with age and delayed surgery but not correlating with other factors such as choledochal exploration, presence of diabetes, or obesity (Tables 11–13).

Discussion

The controversy over the proper timing of intervention in acute cholecystitis has centered around emergency versus elective surgery. In this study, we have chosen an intermediate solution, namely, early surgery within 48 hours of admission to the surgical ward. The 48-hour period was used for preparation and adequate diagnostic investigation. We have come to rely almost completely on ultrasonography in the diagnosis of acute cholecystitis. This method is rapid and noninvasive and has virtually eliminated the laparotomy with false diagnosis of acute cholecystitis.

Definitive surgery was performed in all cases, and temporary cholecystostomy was avoided. Operative cholangiography has proven particularly valuable in the management of acute cholecystitis. The edema around the vital structures renders the palpation of stones difficult and makes the common bile duct and hepatic artery prone to injury. The routine use of operative cholangiography under emergency conditions minimizes the risks of the operation and remains the only practical and reliable guide for formal choledochal exploration. With proper fluorocholangiographic equipment and the skill gained by routine use, operative cholangiography in acute cases is neither difficult to perform nor time-consuming and is of great clinical value.

In our view, the results of this study, in particular the low mortality rate, low incidence of re-

TABLE 7

Choledochal Explorations and Choledocolithiasis, 366 Patients with Acute Cholecystitis

	Cases	%
Choledochal exploration	108	29.5
Choledochal stones	81	22.1
Choledocoduodenostomy	22	6.1

TABLE 8

Gall Bladder Bile in 366 Patients with Acute Cholecystitis and Bacterial Isolates in 211 Positive Bile Samples

	No.	%
Bile findings		
Sterile	122	33.3
Undetermined	33	9.1
Positive	211	57.6
Strains isolated		
E. coli	124	46.6
Klebsiella sp.	46	17.1
Proteus and Pseudomonas	35	13.1
Enterobacter sp.	33	12.4
Enterococci	10	3.8
Salmonella	3	1.1
Bacteroides sp. (anaerobic)	4	1.5
Misc.	11	4.5
TOTAL	266	100

TABLE 9

Sensitivity to Antibiotics (% of Sensitive Stains), 366 Patients with Acute Cholecystitis

	E. coli	Klebsiella	Misc. gram-neg bacteria	Enterococci	All isolates
Ampicillin	55	19	17	75	40
Piparacillin	75	47	51	90	69
Chloramphenicol	60	32	38	50	51
Cephalotin	71	45	28	0	59
Cefoxitin	88	87	54	33	81
Cefamandole	92	81	55	16	82
Cefotaxim	98	96	81	33	91
Gentamicin	92	96	81	0	86
Tobramycin	90	96	83	0	87
Amikacin	88	98	92	0	88

TABLE 10

Postoperative Complications, 366 Patients with Acute Cholecystitis

	No.	%
Wound infection	48	13.1
Respiratory	35	9.5
Miscellaneous	63	17.2
Operative injury to choledocus	0	0

TABLE 11

Mortality in Early versus Delayed Surgery in 366 Patients with Acute Cholecystitis

	No. of Deaths	%
Early (before 72 hrs)	2/294	1
Delayed (after 72 hrs)	2/72	2.8
	p 0.001	

TABLE 12

Postoperative Mortality in 366 Patients with Acute Cholecystitis (4 cases 1.1%)

	No. of pts	%
Age		
Below 70	2.292	0.7
70 & above	2.74	2.7
	p 0.01	
Causes of Death		
Acute myocardial infarction	2	
Acute gastrointestinal bleeding & M.O.F.	1	
Sepsis, burst abdomen	1	

TABLE 13

Statistically Significant Factors Differentiating Chronic and Acute Cholecystitis in 1,804 Patients, 1970–1982

	Cholecystitis		
	Acute (N = 366) %	Chronic (N = 1,438) %	p
% Females	60.1	73.4	0.01
% Patients above 60	51.1	42.3	0.01
% Diabetes mellitus	19.4	10.6	0.001
% Icterus	23.2	13.5	0.001
% C.B.D. stones	22.2	12.4	0.001
Positive cultures	57.6	25.1	0.001
Septic complications	13.1	9.2	0.02
Mortality	1.1	0.4	N.S.

sidual stones, and absence of traumatic injuries to the common bile duct, support the therapeutic policy we have recommended. The management of acute cholecystitis by early surgery and routine operative cholangiography leads to decreased operative mortality and morbidity, decreased hospitalization, and better long-term results.

The bacteriology of bile was extensively studied and sensitivity to newer antibiotics was computed. As in previous publications (10–11), *E. coli* and gram-negative bacilli predominated. Enterococci as well as anaerobic organisms were an uncommon finding (Table 6).

Aminoglycosides as well as the second and third generation cephaloridines were almost equally effective, covering more than 80% of isolates. The short-term perioperative coverage is both useful and cost-effective in acute cholecystitis and an effective drug or combination should be chosen. A combination used in this series, genatamicin plus chloramphenicol, provides an effective and inexpensive solution.

Although certain aspects of therapeutic policy in acute cholecystitis may only be solved by randomized studies, others, such as the use of cholecystostomy, defy true randomization for medical and ethical reasons. Aware of this limitation, we are nevertheless convinced that the results reported favor the therapeutic policy of early and definitive surgery in management of acute cholecystitis.

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The Function of the Autonomic Nervous System and Nonautonomic Components in Juvenile Patients with Borderline Hypertension

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Abstract

The function of the autonomic nervous system and nonautonomic components of juvenile borderline hypertensive patients (ranging in age from 17 to 25 years) without cardiovascular complications were studied by blocking of the autonomic nerve and dynamic exercise. Patients were subdivided into two groups according to their resting cardiac index. (1) During exercise, in the patients with high cardiac output and normal peripheral resistance, vascular resistance did not decrease as much as in the control patients. However, after the administration of phentolamine, peripheral resistance during exercise was reduced significantly and residual vascular resistance became similar in the patient and control groups. In the patients with normal cardiac output and increased peripheral resistance, vascular resistance during exercise was significantly higher than in the controls, as it was before exercise. After the administration of phentolamine, peripheral vascular resistance during exercise was also lowered in these patients. But the residual total peripheral vascular resistance was slightly higher in the patients than the controls. This fact suggests that elevated peripheral resistance during exercise in high- and normal-output hypertension may be maintained by increased alpha-adrenergic vasoconstrictor tone. (2) After the administration of phentolamine, propranolol, and atropine, total peripheral resistance was reduced in the patients with normal cardiac output, but residual resistance remained higher than in the controls, whereas no significant difference was observed in the patients with high cardiac output, and residual resistance became similar to that in the controls. This fact suggests that not only the autonomic nervous system, but also nonautonomic components, may play an important role in maintaining increased peripheral vascular resistance in patients with normal cardiac output and increased peripheral resistance.

Studies of borderline hypertension (1, 2) have attracted the interest of many investigators since such studies may give us information valuable in the early prediction of established hypertension (3–8). Recently, several investigators have suggested that juvenile patients with borderline hypertension have an abnormality of the autonomic nervous system control of circulation (9–18) and display two hemodynamic patterns (19–22). However, the detailed mechanisms of such an abnormality have not yet been clarified. In view of these considerations, I attempted to evaluate the autonomic nervous system control and nonautonomic components in juvenile borderline hypertension by the dynamic exercise test with a bicycle ergometer and blocking of the autonomic nerve with phentolamine, propranolol, and atropine.

Materials and Methods

Eighteen patients with untreated borderline hypertension and ten age-matched healthy male

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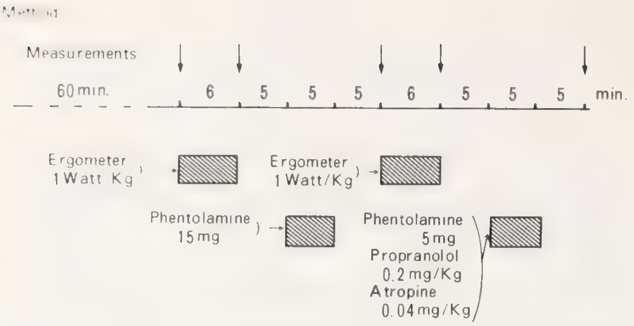

Fig. 1. Schematic presentation of protocol of present study. In each experimental period, measurements of cardiac index, blood pressure, and heart rate in each subject were made.

normotensisve volunteers were studied. Their ages ranged from 17 to 25 years. The average age of the patients was 20.8 ± 0.5 years (mean ± SE) and that of the controls was 20.5 ± 0.5 years. The patients were selected on the basis of having arterial blood pressure readings higher than 150 mm Hg systole or 90 mm Hg diastole at least twice and lower than 150 mm Hg systole or 90 mm Hg diastole at least once out of five consecutive measurements taken at 1-week intervals in the outpatient clinic. All patients were asymptomatic and underwent a full physical examination before the study to eliminate all known causes of secondary hypertension, such as hyperthyroidism, primary aldosteronism, renal artery stenosis, pheochromocytoma, or Cushing's syndrome. Clinical investigations undertaken on the patients included urinalysis, complete blood count, serum and urine electrolytes, intravenous pyelography, creatinine clearance, plasma renin activity, 24-hour urine for VMA, 17-KS, and 17-OHCS, thyroid hormones, chest X-ray, and electrocardiogram. After repeated measurements, all control subjects were found to have blood pressures below 140 mm Hg systole and 90 mm Hg diastole.

The nature of the investigation was explained to all subjects and their prior informed consent was obtained. The subjects were brought to the hemodynamic laboratory after all-night fasting, and the studies were performed with the patient in a supine position without sedatives. A venous cannula was inserted into an antecubital vein, using 0.5% lidocaine anesthesia, for the injection of indocyanine green dye, and an 18-gauge intraarterial cannula was inserted into a brachial artery for the measurement of intraarterial pressure and withdrawal of blood for indicator dilution curves. Cardiac output was measured by the dye dilution technique using a Waters cuvette and a densitometer (Model CoR 100 Cardiac Output Recorder): 2.5 mg of indocyanine green dye was injected into the antecubital vein, which was immediately flushed with a bolus injection of normal saline, 20 ml. Arterial pressure was monitored continuously through a cannula using a Gauld Stetham P23D6 transducer.

The following calculations were made:

mBP = (systolic BP − diastolic BP)/3 + diastolic BP in mm Hg
CI = cardiac output/body surface area in $L/min/m^2$
SI = CI/HR in $ml/beat/m^2$
TPRI = (mBP × 79.92)/CI in $dyne.sec.cm^5.m^2$

where mPB is mean blood pressure, CI is cardiac index, SI is stroke index, HR is heart rate, and TPRI is total peripheral resistance index.

The protocol of this study is shown in Fig. 1. Measurements of the CI, HR, mBP, and TPRI were undertaken in each subject under the following conditions:

1. Resting recumbent 60 minutes after placement of the cannulas.
2. During the sixth minute of dynamic exercise.
3. Five minutes after intravenous administration of phentolamine, 15 mg.
4. During the sixth minute of dynamic exercise, after intravenous administration of phentolamine.
5. Five minutes after additional administration of phentolamine, 5 mg, propranolol, 0.2 mg/kg, and atropine, 0.04 mg/kg.

Phentolamine, propranolol, and atropine were injected for 5 min at a constant speed. After each exercise period, a 15-min interval was provided. Dynamic exercise was performed in a supine position on a Collins bicycle ergometer at a load of 1 watt/kg for 6 min. The analysis of variance and standard t tests were used for statistical analysis of the data. The results were considered significant when p was less than 0.05.

Results

Hemodynamic characteristics of the subjects were studied.

The CI was elevated in a sizable number of the patients with borderline hypertension, but not in all. The average resting CI of the patients was 4.1 ± 0.7 $L/min/m^2$ (mean ± SD) and that of controls was 3.5 ± 0.4 $L/min/m^2$. Therefore, the patients were divided into two groups, those having a CI at least 4.1 $L/min/m^2$ forming the high-car-

TABLE 1

Hemodynamic Characteristics (mean ± SD)

	no.	Age (years)	Heart rate (beats/min.)	Blood pressure (mm Hg)	Mean blood pressure (mm Hg)	Cardiac index (L/min./m^2)	Stroke index (ml/beat/m^2)	Total peripheral resistance index (dyne · sec · cm^{-5} · m^2)
Controls	10	20.5 ± 1.5	64 ± 5	110 ± 10 67 ± 8	80.9 ± 7.9	3.5 ± 0.4	54.9 ± 7.8	1881 ± 137
High-output patients	9	20.7 ± 1.7	77 ± 5***	137 ± 6*** 88 ± 6***	104.8 ± 5.4***	4.7 ± 0.4***	60.7 ± 5.4	1801 ± 117
Normal-output patients	9	21.6 ± 2.1	68 ± 4	132 ± 8*** 87 ± 8***	102.2 ± 6.4***	3.5 ± 0.2	52.0 ± 4.3	2297 ± 147***

The cardiac index (4.1 L/min/m^2) was used to define the high and normal cardiac groups. Significance levels of difference between control subjects and borderline hypertensive patients (*) and between normal- and high-cardiac-output borderline hypertensive patients (+): $^{++}$ = $p < 0.01$; *** = $p < 0.001$. n = number of subjects.

diac-output hypertension group and the remainder forming the normal-cardiac-output hypertension group.

The hemodynamic characteristics of the patients classified as high- and normal-cardiac-output borderline hypertension subjects and those of the normal controls are shown in Table 1.

High-Cardiac-Output Hypertension

The TPRI of the patients and the controls was similar. Systolic, diastolic, and mean arterial pressures in the patients were significantly increased compared with the controls at $p < 0.001$, $p < 0.001$ respectively. The HR was significantly higher in this group than among the controls ($p < 0.001$) or the patients with normal cardiac output ($p < 0.01$). In addition, the SI was greater in this group than in the patients with normal cardiac output ($p < 0.01$).

Normal-Cardiac-Output Hypertension

The TPRI was greater in this group than in the controls ($p < 0.001$) or the patients with high cardiac output ($p < 0.001$). Systolic, diastolic, and mean arterial pressures in this group were significantly increased compared with the controls at $p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively, but there was no difference between this group and the patients with high cardiac output. The HR in this group was not significantly different from that of the controls ($p < 0.05$). In the CI and SI, however, there was no difference between this group and the controls.

Hemodynamic Response to Exercise Before and After Administration of Phentolamine

High-Cardiac-Output Group. During exercise, the TPRI was reduced significantly in both the controls ($p < 0.01$) and the patient group ($p < 0.01$), but the degree of TPRI reduction in the patient group was not as steep as that observed in the controls. Therefore, the TPRI during exercise was significantly higher in this group than in the controls ($p < 0.001$). Administration of phentolamine reduced the TPRI to the same extent in both the patient and control groups. After intravenous administration of phentolamine, exercise reduced the TPRI significantly in patients with high cardiac output ($p < 0.01$) but did not reduce it significantly in the controls. Consequently, the difference in residual TPRI between the patient group and the controls was abolished with exercise (Fig. 2).

Normal-Cardiac-Output Group. During exercise, the TPRI was reduced significantly ($p < 0.01$) compared with the resting value in both the control and the patient groups, and the degree of reduction was the same as in the controls. The TPRI during exercise remained at a higher level than in the controls ($p < 0.001$). After administration of phentolamine, the TPRI was reduced in the patient group ($p < 0.01$) as well as in the controls ($p < 0.01$), and the residual TPRI was still higher in the patients than in the controls ($p < 0.01$). After administration of phentolamine, exercise reduced the TPRI significantly in the patient group, but the reduction in the controls was not significant. So the residual TPRI was slightly higher in the patients than in the controls ($p < 0.05$) (Fig. 3).

Hemodynamic Response After Administration of Phentolamine Combined with Propranolol and Atropine (Total Autonomic Block). No significant changes of the TPRI produced by total autonomic block were observed either in the patients with high cardiac output or in the controls. The residual TPRI was similar in patients and controls.

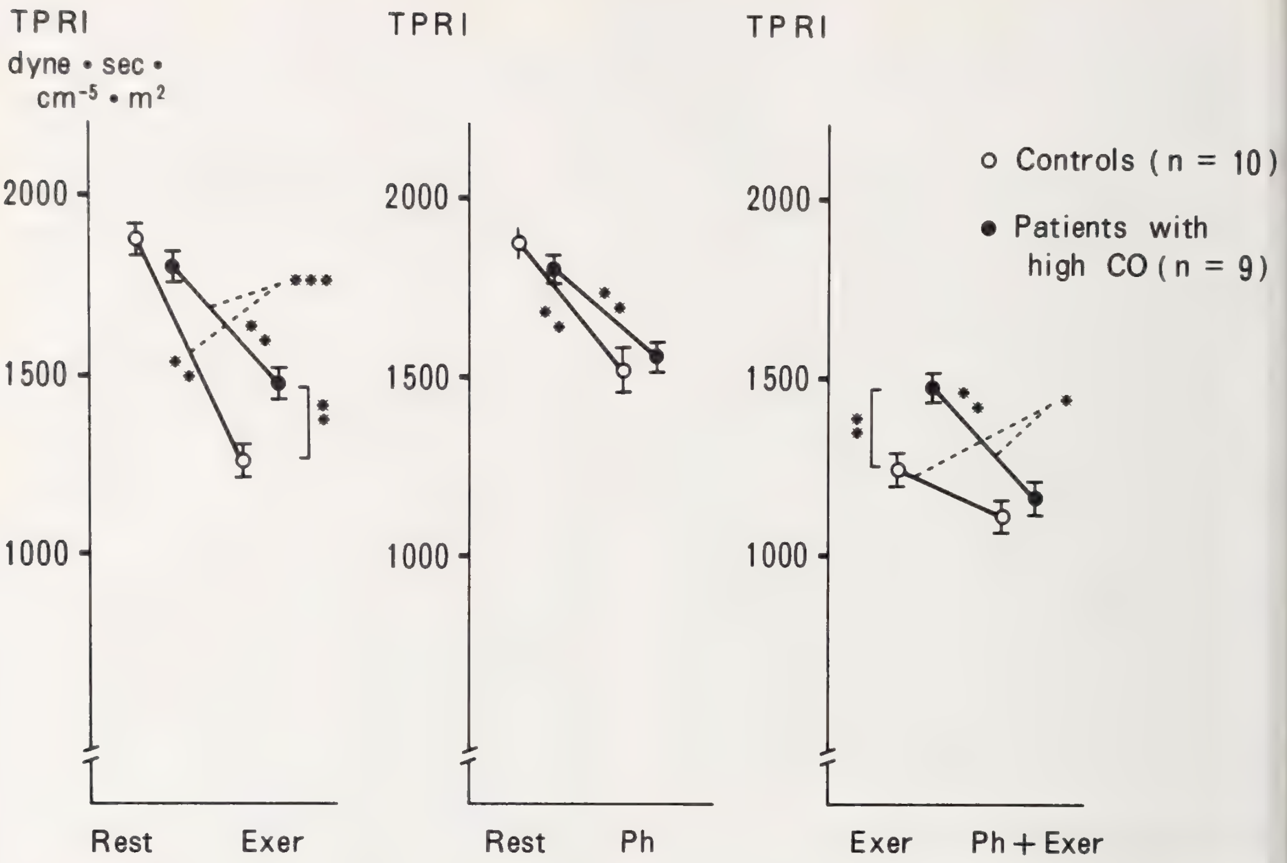

Fig. 2. Total peripheral resistance index at rest, during exercise, after administration of phentolamine, and during exercise after administration of phentolamine in patients with high-cardiac-output hypertension (closed circles) and in normal control subjects (open circles). Exer, exercise; Ph, phentolamine. During exercise after administration of phentolamine, no significant difference of residual peripheral resistance in patients and controls was observed.

However, in the normal-cardiac-output group, the TPRI at rest was higher than in the controls ($p < 0.001$). After total autonomic block with the three drugs, the TPRI was reduced significantly ($p < 0.05$) only in the normal-cardiac-output group. However, the residual TPRI in this group remained higher than in the controls ($p < 0.01$) or in the patients with high cardiac output ($p < 0.01$) (Fig. 4).

Discussion

Abnormality of Total Peripheral Resistance

During Exercise. In established hypertension, the cardiac output is normal and the peripheral resistance is elevated (22, 23). On the other hand, in a substantial proportion of patients with borderline hypertension, cardiac output is elevated at rest, but this has not been found in all patients (24, 25); in some cases, cardiac output is normal.

Safer et al (25) attempted to divide patients with borderline hypertension into two groups according to their resting cardiac output. In this study, I classified patients with borderline hypertension in the same way as Safer and examined the hemodynamics of these patients. There were two patterns of resting hemodynamics among the patients with borderline hypertension: (a) high cardiac output with increased HR and normal peripheral resistance and (b) normal cardiac output with somewhat increased HR and increased peripheral resistance. These hemodynamic characteristics of borderline hypertension were similar to those reported previously (19–22, 26). The TPRI was normal at rest in the patients with high cardiac output, but during exercise, it was higher than in the controls. Similar findings have been reported by previous investigators (26, 27). If a stimulus requiring a decrease of peripheral resistance is given, patients with borderline hypertension may fail to show a decrease in peripheral resistance to the same degree as normotensive controls (28).

Safer et al (25) subdivided borderline hypertension patients into "high-output" and "normal-output" groups. Among patients with high cardiac output and normal resistance, diastolic orthostatic hypotension was observed and the total

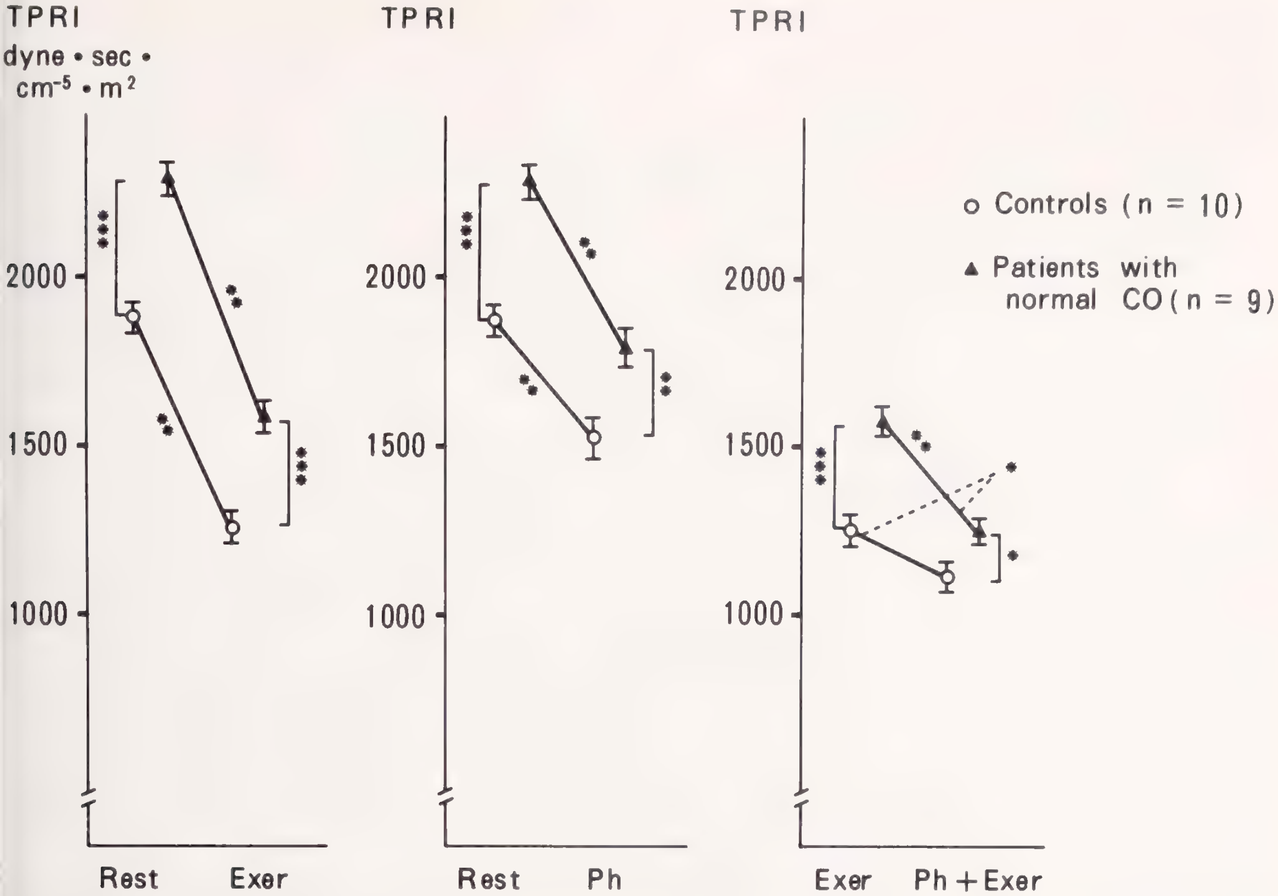

Fig. 3. Total peripheral resistance index at rest and after various loads shown in Fig 1 in the patients with normal-cardiac-output hypertension (triangles) and in normal control subjects (open circles). Exer, exercise; Ph, phentolamine. Mean values ± SE of mean are shown. Significance of difference is denoted by asterisks (*** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$). Total peripheral resistance index during exercise was higher in patients than in controls, but after phentolamine infusion was at the same level in both groups.

peripheral resistance was greater than in the controls despite an abnormal fall in the CI, whereas the hemodynamic response to exercise indicated that total peripheral resistance during exercise was not reduced as it was in the controls and patients with normal cardiac output. Further studies were performed, resulting in the suggestion that the elevated blood pressure in the high-cardiac-output patients may stem from an inappropriate adjustment of the peripheral resistance to the increased tissue perfusion accompanying the increased cardiac output.

In this study, the change in total peripheral resistance produced by exercise was examined before and after administration of phentolamine, which is known to be an alpha-adrenergic blocking drug. During exercise before the administration of phentolamine, peripheral resistance in the patients with high cardiac output was not reduced to the same degree as in the controls. However, during exercise after the administration of phentolamine, total peripheral resistance was significantly reduced in the patients with high cardiac output. Therefore, residual peripheral resistance in the patients reached the same level as in the controls. It appears that the elevated total peripheral vascular resistance during exercise may be maintained by increased alpha-adrenergic vasoconstrictor tone in patients with high cardiac output. Total peripheral resistance at rest was high in the patients with normal cardiac output. During exercise, total peripheral resistance was reduced to the same degree as in the controls, but residual resistance among the patients still remained at a higher level than in the controls. During exercise after the administration of phentolamine, total peripheral resistance in these patients was significantly reduced. Consequently, the residual TPRI in this group decreased very close to that in the controls; the former, however, still remained slightly higher than the latter. It appears that in patients with normal cardiac output, too, some part of the elevated total peripheral resistance during exercise

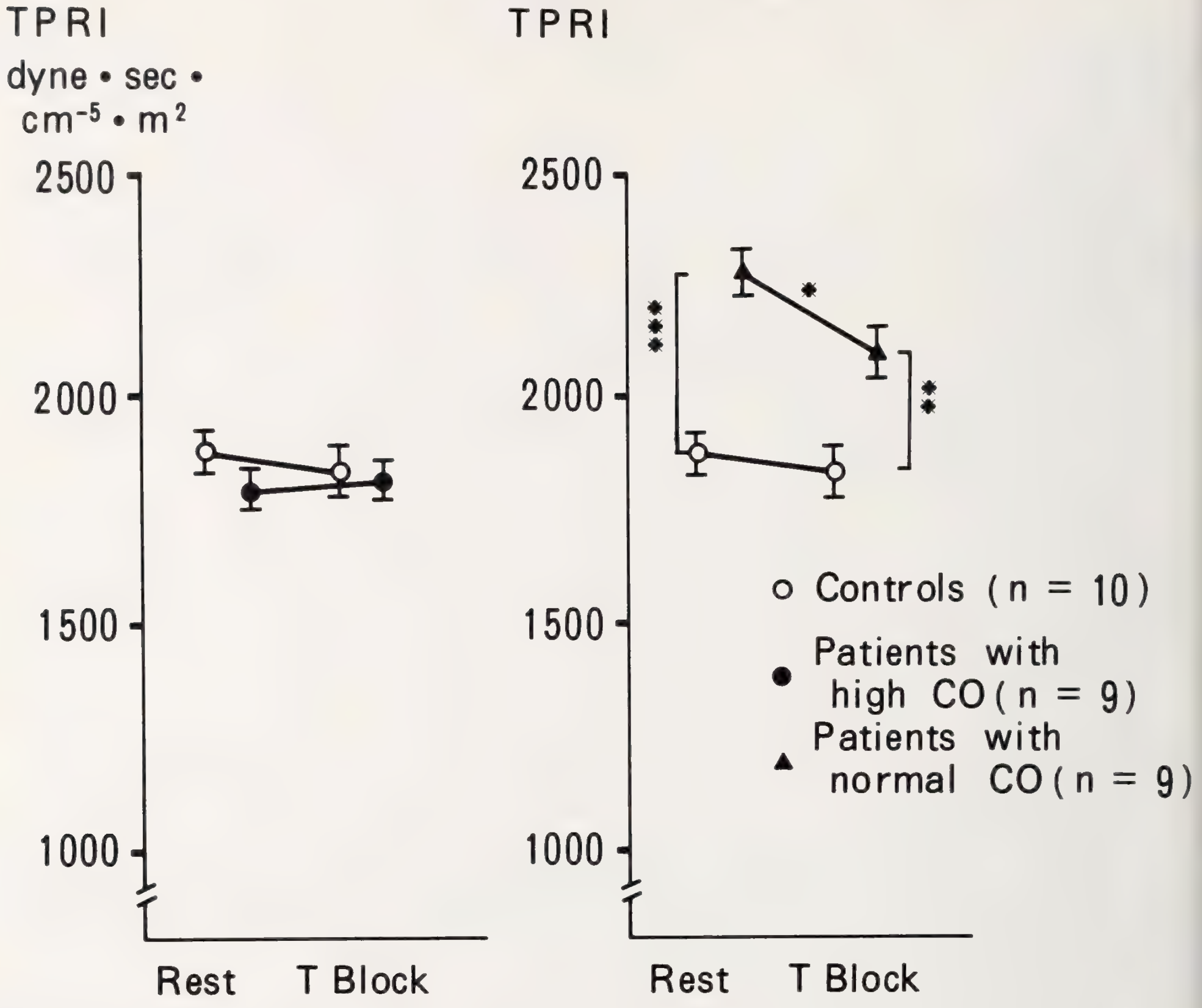

Fig. 4. Total peripheral resistance index after administration of phentolamine, propranolol, and atropine in patients with high-output and normal-output hypertension as well as in control subjects. Symbols as in Fig. 2. T Block, total block by administration of phentolamine, propranolol, and atropine. After total block, total peripheral resistance index was significantly higher in patients with normal cardiac output than in controls.

may be maintained by increased alpha-adrenergic vasoconstrictor tone.

After Administration of Phentolamine, Propranolol, and Atropine. Pharmacologic autonomic blockade by the administration of propranolol, atropine, and phentolamine has been used to evaluate the roles of autonomic and nonautonomic components in hemodynamic abnormalities of patients with hypertension. The dose of drugs used in this study was similar to those used in previous studies (28–31). The doses of propranolol (32) (0.2 mg/kg), atropine (33) (0.04 mg/kg), and phentolamine (30, 31) (15 mg) have been proven to be sufficient to block adrenergic and parasympathetic effects by previous investigators. Tanaka et al (30) observed hemodynamic abnormalities in patients with mild hypertension after administration of autonomic blockade and suggested that nonautonomic components play an important role in maintaining increased peripheral vascular resistance in borderline hypertension.

In this study, patients with borderline hypertension were subdivided into two groups and examined. Total peripheral resistance in the patients with high cardiac output, originally not greatly different from that of the controls, came even closer to the latter after administration of phentolamine, propranolol, and atropine. However, in the patients with normal cardiac output, residual peripheral resistance after total autonomic block remained at a higher level than in the controls or the patients with high cardiac output.

Such abnormal residual resistance after total autonomic block, as observed in the patients with normal cardiac output, may be due to nonautonomic components, such as structural changes, as well as to established hypertension. If so, the latter hemodynamic characteristics in patients with normal cardiac output may represent a somewhat later stage of the hypertension process.

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A Qualitative Analysis of the Frequency Response Characteristics of a Hydraulic Esophageal Manometry System

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Abstract

A qualitative frequency response analysis of a multilumen hydraulic–capillary infusion system used for esophageal motility assessment reveals substantial variation in the natural frequency and damping coefficient among the channels of the multilumen catheter. However, the frequency response is relatively independent of the infusion rate and is flat to a frequency of about 4 Hz, thus permitting a virtually distortion-free recording of the slow peristaltic contractions. The presence of an air bubble in one channel has, as expected, a profound deleterious effect on the frequency response characteristics of that channel, severely distorting or obliterating the transmission of relatively fast esophageal spasms; however, slow peristaltic contractions may be transmitted along this overdamped channel with adequate fidelity.

In the multilumen, hydraulic–capillary infusion system used in esophageal manometry, the esophageal pressure fluctuations detected at the distal orifice of the manometry catheter are transmitted along the water column to a bank of pressure transducers and thence to a multichannel pen recorder. The fidelity with which the pressure fluctuations that are detected at the orifices is reproduced on the recorder is a function of the frequency response characteristics of the "transmission line" that transmits the pressure fluctuations along the water column to the transducers and ultimately to the recording pens.

Any time-varying signal such as a simple square wave, an arterial pressure wave, or a peristaltic pressure wave can be mathematically reconstructed with theoretically infinite fidelity by the judicious superposition of an infinite series of sinusoidal "harmonic" waves of differing frequency, amplitude, and phase (1). In order to reproduce a time-varying signal with an acceptable degree of fidelity, the detection mechanism, transmission medium, and recording apparatus must be capable of detecting, transmitting, and recording an acceptable bandwidth of sinusoidal waves. A system (detection mechanism, transmission medium, and recording apparatus) that is to reproduce fast time-varying signals must be capable of processing higher sinusoidal frequencies, i.e., must have a higher-frequency response, or a wider bandwidth, than a system that is to reproduce slow signals. Thus the reproduction and recording of the relatively fast pressure fluctuations occurring in the cardiovascular system require a wider bandwidth with a higher-frequency response (2) than do the reproduction and recording of the relatively slow pressure fluctuations encountered in the peristaltic activity of a normal esophagus. When fast, sharp pressure spikes are transmitted over a system with a limited high-frequency response, the pressure spikes are recorded as prolonged, dampened, and rounded pressure waves; an esophageal manometry system with a limited frequency response would faithfully reproduce the slow peristaltic contractions, but the faster esophageal spasms would be recorded with distorted and significantly diminished peak amplitudes.

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Materials and Methods

The manometry catheter is a PVC assembly whose outside diameter measures 4.5 mm, comprising eight lumens each with an inside diameter of 0.8 mm, circumferentially distributed around a 1.6-mm-diameter center lumen (Arndorfer Medical Specialties, Greendale, WI). The distal end of each of the eight circumferential lumens terminates in an 0.8-mm-diameter side orifice; the eight orifices are longitudinally spaced at 3-cm intervals. The proximal end of each circumferential lumen is terminated in a female Luer fitting that is mated to a pressure transducer, Beckman Type 4-327-C. Hydraulic pressure is furnished by a hydraulic–capillary infusion system (Arndorfer Medical Specialties, Greendale, WI) that allows the hydraulic pressure at the transducers to be regulated from 0 to 30 psi (Fig. 1). The outputs from the transducers are recorded on a Beckman R 611 multichannel recorder system with Type 9853H couplers.

The variable-frequency pressure generator (Fig. 2) was designed and constructed by a group of senior mechanical engineering students from the New Jersey Institute of Technology. The distal end of the manometry catheter with the linear array of pressure-sensing orifices is sealed into a cylindrical plexiglass pressure chamber with conventional clay. One pressure transducer is connected directly to the chamber to furnish a reference pressure signal. The pressure in the chamber is varied sinusoidally by a bellows pump which is coupled through a Scotch yoke to a variable-speed electric motor. The frequency of the sinusoidal pressure oscillations in the chamber is manually adjustable from 2 Hz to approximately 26 Hz; peak-to-peak pressures measure approximately 50 mm Hg.

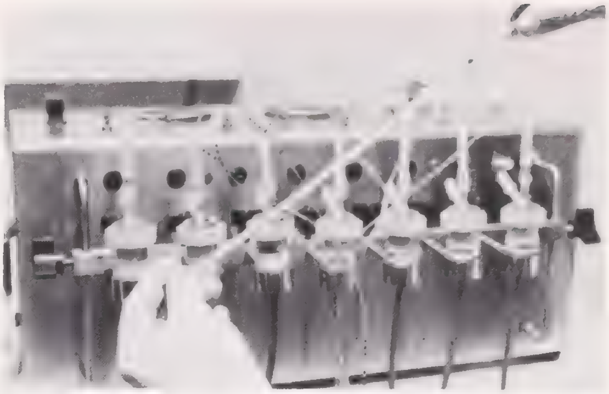


Fig. 1. Manometry catheter, with pressure transducer bank and hydraulic–capillary infusion system in background.

Results

With the manometry catheter sealed into the plexiglass pressure chamber, the frequency of the pressure oscillations was manually increased from about 2 Hz to a maximum of 26 Hz and then decreased again to the minimum frequency. The output from the reference transducer that measures the pressure in the pressure chamber was recorded on channel 1 of the multichannel recorder. Channels 2–7 record the pressures detected at six of the eight pressure-sensitive orifices of the manometry catheter.

Figure 3A–C depicts the qualitative frequency response characteristics of the six lumens of the manometry catheter. The differences between

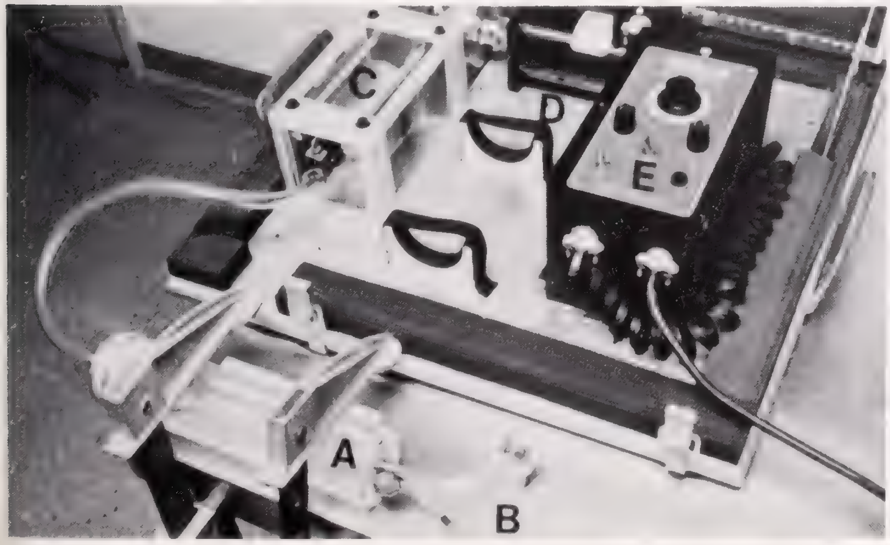
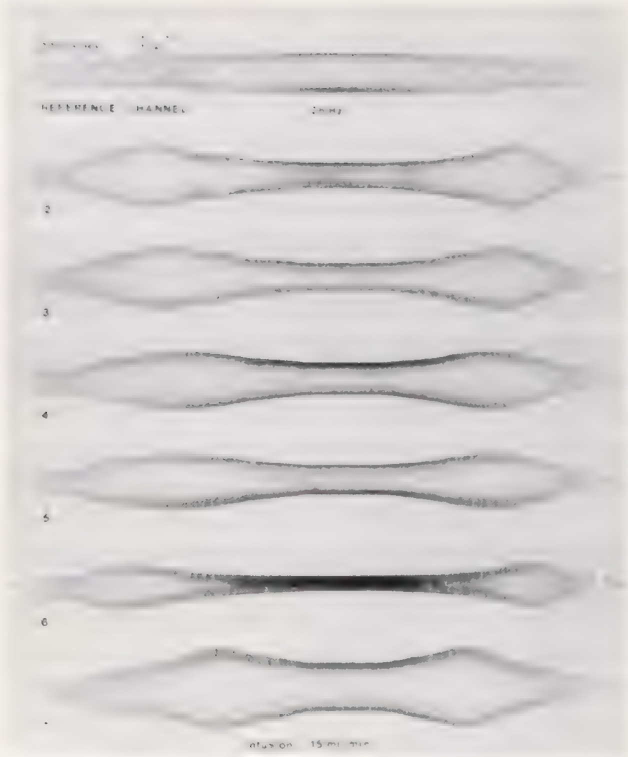
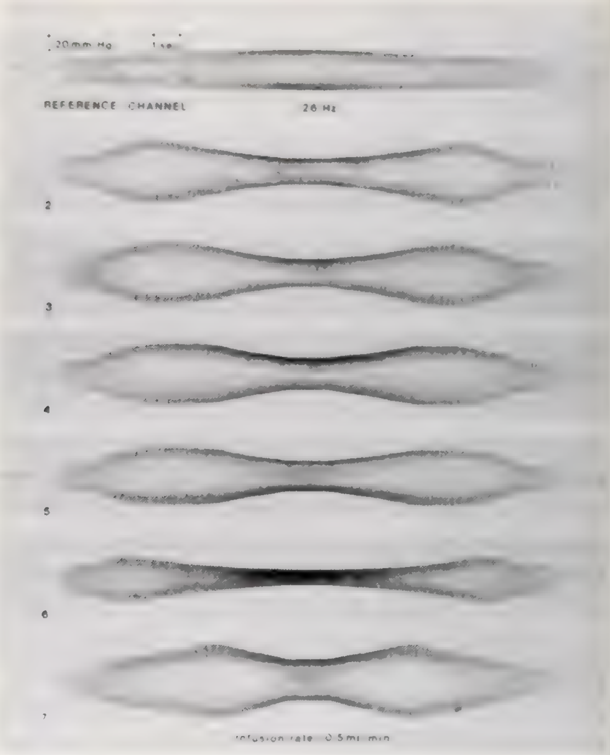


Fig. 2. Variable-frequency pressure generating apparatus. (**A**) Plexiglass pressure chamber with manometry catheter. (**B**) Reference transducer. (**C**) Bellows pump. (**D**) Variable speed motor. (**E**) Motor control.





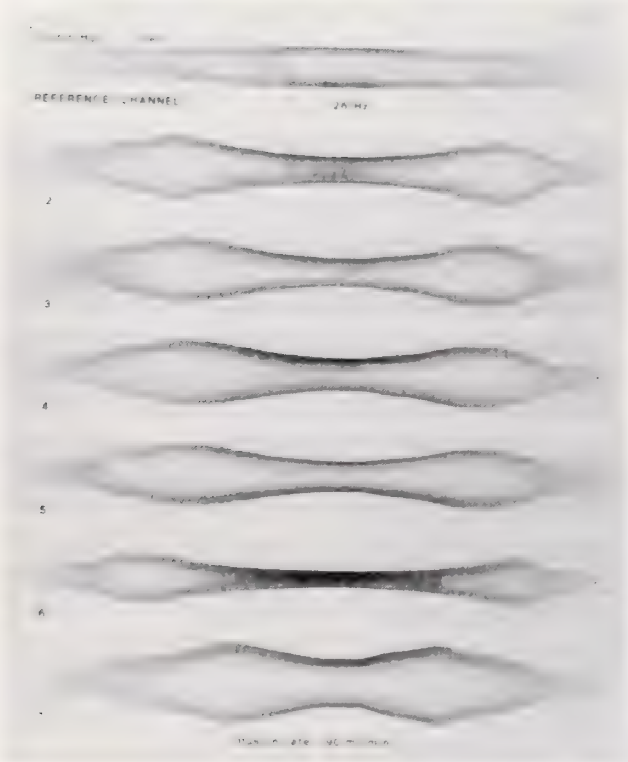


Fig. 3A. Top left Frequency response at infusion rate of 0.15 ml/minute.
Fig. 3B. Top right Frequency response at infusion rate of 0.50 ml/minute.
Fig. 3C. Bottom left Frequency response at infusion rate of 0.90 ml/minute.

each of the tracings on channels 2–7 and channel 1 reflect the contribution of the hydraulic infusion manometry system to the frequency response of the entire system. Figure 3A–C depicts the differing infusion rates at which water flows through the lumen and out the side orifices; in Fig. 3A the infusion rate is approximately 0.15 ml/minute, in Fig. 3B it is 0.50 ml/minute, and in Fig. 3C it is 0.90 ml/minute.

The differences in the qualitative frequency response patterns of Fig. 3A–C are negligible, leading to the conclusion that the inherent frequency response characteristics of the manometry catheter are not particularly sensitive to the rate at which the water is being infused through the catheter lumens. However, in this apparatus the pressure-sensing orifices of the manometry catheter are subjected to air pressure fluctuations; in the physiologic situation, the esophagus may collapse around the catheter, and the additional surface tension of the tissue might occlude the pressure-sensitive orifices. To increase the infusion rate much beyond that required to prevent occlusion is not warranted.

Mathematically the hydraulic–capillary infusion catheter can be characterized as a "second-order" system (2), that is, a system characterized by a natural frequency and a damping coefficient. In the absence of viscous damping, the system would respond with a large amplitude at its natural frequency, analogous to the oscillation of an ideal, frictionless spring. With the addition of moderate viscous damping, a peak amplitude response will be attained at a frequency somewhat lower than the natural frequency. Once a "critical" damping value is exceeded, the response of the system will decay continuously as the frequency is increased (1).

A qualitative examination of the frequency response patterns of Fig. 3 reveals that the natural frequency and damping coefficient of channels 2–5 are quite similar. These channels attain a maximum response of 1.6–2.0 times the amplitude of the reference channel 1, at a frequency of roughly 14 Hz. The viscous damping in channel 6 is the highest of the six lumens with a maximum response of 1.4 times that of the reference channel, at a frequency of roughly 10 Hz. Channel 7 with the smallest damping coefficient responds with an amplitude of about 2.2 times the reference channel amplitude, at a frequency of about 18 Hz.

The frequency response of all six channels is flat and equal to that of the reference channel, up to a frequency of about 4 Hz. This is a more-than-adequate response to reproduce, with negligible distortion, the normal, smooth, and relatively slow peristaltic contractions encountered in even a moderately spastic esophagus.

For the frequency response patterns in Fig. 4, a small, roughly 0.1 cc, air bubble was deliberately introduced into the dome of the pressure transducer of channel 4. The infusion rate was set at 0.5 ml/minute. Unlike water, air is a compressible fluid and hence functions as a "shock absorber" to absorb pressure fluctuations transmitted from the pressure-sensitive orifice to the transducer. Channel 4 becomes heavily overdamped, resulting in a frequency response that decays rapidly, beginning at the low frequency, to a negligible response.

To simulate esophageal spasms, the bellows pump that generates sinusoidal pressure fluctuations in the pressure chamber was replaced by a conventional bicycle pump. With sharp thrusts on the pump plunger, the pressure fluctuations of Fig. 5 were recorded. As in Fig. 4, channel 4 was overdamped by the air bubble in the dome of the transducer; the infusion rate was set at 0.5 ml/minute. The "spastic" pressure fluctuations are almost entirely obliterated in the overdamped channel 4; however, the slow pressure variations are still reproduced with acceptable fidelity. The amplitude of the spastic fluctuations in the remaining channels is magnified by the frequency response maxima illustrated in Fig. 3; the magnification factor ranges from 1.2 to 1.5 relative to

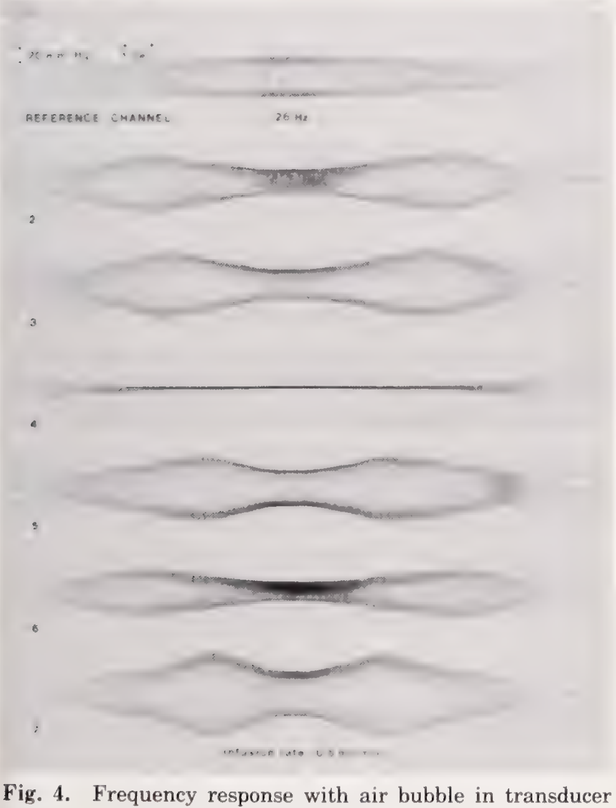


Fig. 4. Frequency response with air bubble in transducer dome of channel 4. Infusion rate is 0.50 ml/minute.

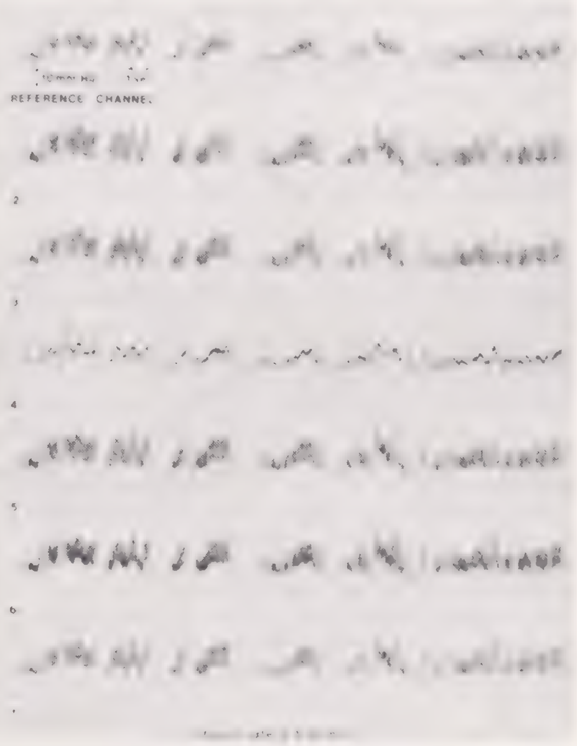


Fig. 5. Reproduction of simulated spasms. Air bubble is present in transducer dome of channel 4. Infusion rate is 0.50 ml/minute.

the amplitude of the spasms recorded in reference channel 1.

Summary

The interpretation of pressure recordings from an esophageal motility evaluation is more a qualitative assessment of the peristaltic response to deglutition than a precise quantitation of the amplitude of the peak peristaltic pressures. Consequently, overconcern with the frequency response characteristics of the instrumentation system is not necessary. As long as the pressure-sensitive orifices of the hydraulic manometry catheter are not occluded, the fidelity of the pressure recordings is quite insensitive to the infusion rate. Even in the presence of an air bubble in the system, the slow peristaltic pressure wave will be transmitted with adequate fidelity; however, the faster esophageal spasm will be significantly distorted and may be dampened out. A periodic inspection of the integrity of the domes and gaskets of the pressure transducers is certainly in order to assure that no air can infiltrate the system.

Acknowledgments

We acknowledge the vital contribution of E. Paul Strauss, Ray Wendrzycki, and John Lutz who, as senior engineering students working under the direction of Prof. Martin J. Linden at the New Jersey Institute of Technology, designed, constructed, modified, and ultimately rendered operational the variable-frequency pressure generator depicted in Fig. 2.

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Status Epilepticus Following Electroconvulsive Therapy

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Electroconvulsive therapy (ECT) is an accepted treatment modality for affective disorders and consists of controlled electrical stimulation with resultant seizure under drug modification (glycopyrrolate, methohexital, and succinylcholine) (1, 2). The electrically induced seizure usually lasts less than 60 seconds (3). ECT, when given as the standard single treatment three times a week, is uncommonly complicated with prolonged seizures (4); with multimonitored ECT, the incidence of this complication increases (5, 6). Status epilepticus following ECT is quite rare but has been reported with single unilateral electrode placement (7, 8), single bilateral electrode placement (9), and multimonitored ECT (10). In this paper we present three cases involving prolonged seizures (more than 200 seconds and requiring benzodiazepine intervention for cessation) and/or status epilepticus (more than 10 minutes consecutive seizure time or initiation of second seizure requiring benzodiazepine intervention for cessation).

Case Reports

Case 1. A 51-year-old white woman was admitted to a private acute general medical center for her third psychiatric hospitalization. Her symptoms included decreased appetite, decreased energy, irregular sleep, self-deprecatory thoughts, decreased concentration, decreased interests, and significant suicidal ideation. During prior hospitalizations, the patient had been treated with phenelzine, amitriptyline, amoxapine, doxepin, trazodone, and thioridazine in combination with psychotherapy, without significant effect. The admitting diagnosis was major affective disorder, depression, recurrent (11).

The patient received the standard ECT workup with appropriate consultations, consents, and medical clearance. No pre-ECT EEG was performed.

The patient was scheduled for three treatments a week, and the first four were given as bifrontals with settings on the MECTA ECT Model C Instrument of 70 (frequency), 1.5 milliseconds (pulse width), and 2.0 seconds (stimulus duration). No complications were noted. The patient was concurrently on trazodone 200 mg, ranitidine 150 mg, thioridazine 25 mg, hyoscyamine 0.125 mg a half-hour before meals, and calcium/vitamin supplements. The fifth ECT was changed to right unilateral without change in medications, ECT premedications, or power settings. The treatment was complicated by an extended seizure lasting 15 minutes; the seizure was controlled with diazepam intravenous push in 5 mg/5 minutes ×3 or 15 mg. The patient had one further ECT, again of right unilateral placement, and on this occasion the patient had a grand mal seizure ×1 of 40 seconds followed by a series of grand mal seizures persisting for 35 minutes; a total of 30 mg diazepam intravenous push was required to eventually control status epilepticus. At that point ECT was discontinued.

CT scan with and without contrast was interpreted as negative. The EEG done directly after the last ECT was abnormal, reflecting non-

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specific diffuse cerebral dysfunction with frontal intermittent rhythmic delta activity. A repeat EEG 6 days later was considered improved. The patient was placed on carbamazepine prophylactically and a third EEG, 20 days after ECT, revealed bitemporal spike foci with right midtemporal predominance.

The patient was readmitted 4 months later for a depressive decompensation, at which time the admission plan was a repeat ECT series while on prophylactic carbamazepine. Blood levels were maintained between 4.4 μg/ml and 5.5 μg/ml on carbamazepine 200 mg twice a day. Concurrent medications included thioridazine 25 mg daily, protriptyline 10 mg three times a day, fludrocortisone 0.1 mg and ranitidine 150 mg twice a day, hyoscyamine 0.125 mg a half-hour before meals, and calcium/vitamin supplements. A pre-ECT EEG was interpreted as normal.

The first 3 treatments were performed as right unilaterals, and the last 9 as bifrontals. Power settings were 70 (frequency), 1.5 milliseconds (pulse width), and 2.0 seconds (stimulus duration). A portable EEG with incomplete montage (FP_1, FP_2, T_3, T_4, O_1, O_2) of the first treatment revealed a single seizure of 20 seconds, followed by diffuse slowing and returning to normal pre-ECT baseline without persistent epileptiform activity noted within 10 minutes. No prolonged seizures occurred throughout the series.

Case 2. A 70-year-old white woman was admitted to a private acute general medical center for one of multiple psychiatric hospitalizations. Her symptoms included decreased appetite, decreased energy, decreased sleep, decreased interests, self-deprecatory thoughts, difficulty concentrating with impaired memory, thoughts of death, and even somatic delusions. The admitting diagnosis was major affective disorder, depression, recurrent (11). The patient had previously responded to ECT.

The admitting plan was to attempt a short chemotherapeutic trial prior to ECT. Trazodone was rapidly titrated to 100 mg; however, the patient complained of increased weakness, and borderline tachycardia was noted. The patient continued with severe anorexia and, in concert with the internal medicine physician, it was determined that this patient required rapid intervention.

The standard ECT workup with appropriate consultations, consents, and medical clearance was performed. A pre-ECT EEG revealed "both isolated mildly slow waves of 6–8 Hz and occasionally high amplitude bursts of moderately slow waves of 4–5 Hz; these appeared to be generalized in the common reference electrodes in the scalp and scalp combinations; slow wave activity is definitely more from the left hemisphere than from the right and more from the posterior half of the left hemisphere." The disorganization did not include epileptiform activity.

A CT scan with and without contrast revealed mild cerebral atrophy only. Medical illnesses actively under treatment included a dumping syndrome with intermittent gastritis and duodenitis secondary to multiple abdominal operations (gastric resection with pyloroplasty for gastric carcinoma), arteriosclerotic coronary vascular disease, hypertension, and hypothyroidism. In addition to trazodone, the patient was taking levothyroxine 0.2 mg and digoxin 0.125 mg daily in the morning, pindolol 10 mg twice a day, flurazepam 30 mg at bedtime as necessary, and magaldrate 30 cc as needed for dyspepsia.

ECT was performed as a right unilateral with power settings of 70 (frequency), 1.5 milliseconds (pulse width), and 2.0 seconds (stimulus duration). The first treatment was complicated by persistent seizure activity: "following return to autonomous breathing at 6′36″ the patient, approximately two minutes later, developed generalized tonic-clonic movements in the upper and lower extremities—objectively greater on the right." This additional seizure activity was rapidly controlled with 10 mg diazepam intravenous push. Electrolytes were normal and post-ECT portable EEG revealed diffuse cerebral dysfunction without sharp waves, spikes, focal slowing, or other paroxysmal activity.

The patient was placed on phenytoin and ECT was held until a therapeutic blood level (11.7 μg/ml) was obtained. Trazodone was discontinued.

The second ECT treatment was attempted with an anesthesiologist, a neurologist, and the EEG Department in attendance. No clinical response was noted using the same MECTA power settings, and the patient was changed to a Dale ECT machine. No clinical response was noted at 400–500 milliamps. The portable EEG throughout the attempted second treatment demonstrated no seizure activity.

Phenytoin was discontinued and the blood level dropped to 3.4 μg/ml prior to the third ECT. Again no clinical response was noted with the MECTA; however, a grand mal seizure was clinically observed on the Dale at 450 milliamps. The portable EEG revealed "50 second spike and dome activity generalized throughout both hemispheres without focality and without additional inappropriate post-ECT secondary seizures." With the patient no longer taking trazodone and phenytoin, the ECT series was successfully completed without further complication.

Case 3. A 69-year-old white woman was admitted to a private acute general medical facility for her fourth psychiatric hospitalization. Her symptoms included decreased appetite, energy, sleep, concentration, and interests; self-deprecatory thoughts; suicidal ideation; paranoid delusions; and auditory hallucinations. The admitting diagnosis was major affective disorder, depression, recurrent (11). During prior hospitalizations the patient had been treated with amoxapine, trazodone, fluphenazine, thiothixene, alprazolam, and other psychotropics, in combination with psychotherapy, without significant effect. She had previously improved dramatically with ECT.

The patient received the standard ECT work-up with appropriate consultations, consents, and medical clearance. Pre-ECT EEG was interpreted as normal. Active medical illnesses included chronic lymphocytic leukemia and hypertension treated with methyldopa 500 mg twice a day. At the time of the first ECT, the patient received flurazepam 15 mg at bedtime as the only psychotropic. MECTA power settings were 70 (frequency), 1.5 milliseconds (pulse width), and 2.0 seconds (stimulus duration). The first treatment was complicated by status epilepticus: "ECT #1 was performed as a right unilateral. Initial breathing time was 4′00″. However, at approximately 4′15″, seizure activity was clinically noted. By 6′15″ 10 mg intravenous diazepam was given without cessation of seizure. The EEG was restarted and at 11′15″ a further 10 mg intravenous diazepam was given, with effect. Constant EEG recording noted a cessation of electroencephalographic seizure activity by 13′. The patient was followed with EEG tracings through 17′ and remained in the ECT room for 25′ with no further activity."

CT scan revealed cortical atrophy only. In neuropsychometric testing the patient's score was 7/30 on the Folstein mini-mental status examination (12) and 38 on the Hamilton depression scale (13). Trazodone was rapidly titrated to 250 mg a day and thiothixene similarly to 30 mg a day. No significant improvement was noted; her repeat score on the Hamilton scale was 42 and, on the Folstein, 16/30. Trazodone was discontinued in preparation for further ECT. Phenytoin was given prophylactically.

Using the same power settings, ECT was unsuccessful on two occasions—on the first the phenytoin blood level was 22.0 μg/ml, and on the second the level was 9.8; therefore, phenytoin was discontinued and carbamazepine was given prophylactically. With a phenytoin level of 3.0 μg/ml and a carbamazepine level of 5.4 μg/ml, no clinical seizure occurred with maximal MECTA power settings; however, with a Dale ECT machine at 400 milliamps × 4 second stimulus duration, a 30-second clinical and electrical seizure was noted. Thereafter all anticonvulsants were discontinued. With the patient's carbamazepine level at 5.0 μg/ml, the next ECT was successful, utilizing the MECTA. All further ECT were completed without complication. Further repeat neuropsychometric testing revealed a score on the Hamilton depression scale of 14 and, on the Folstein mini-mental state test, a score of 28/30.

Discussion

Prolonged seizures and status epilepticus following ECT may be related to various etiologic factors, including hyperoxygenation, lowered seizure threshold associated with medications, and preexisting epileptiform activity. In all cases preoxygenation with ventilation occurred throughout the entire procedure without change in technique during the ECT series. In cases 1 and 2 the patients were receiving an antidepressant when post-ECT status epilepticus or prolonged seizures occurred; in case 1 the patient was also receiving a neuroleptic. Most antidepressants have been reported to lower the seizure threshold with spontaneous seizures or cerebral dysfunction (14–16). The antidepressant common to both cases in our series was trazodone, a nontricyclic, non-MAOI antidepressant noted to have minimum anticholinergic side effects (17, 18), efficacy in the geriatric population (19), and only two reports in the literature suggesting lowered seizure threshold (20, 21). In case 1, bitemporal spike foci with right midtemporal predominance was noted on an EEG performed 20 days after ECT. This underlying epileptiform activity may have served as an etiologic factor with trazodone, since the status epilepticus occurred only with *right* unilateral electrode placement. In case 3 no etiologic factor was found.

The presence of prolonged seizures or status epilepticus requires rapid intervention (4, 9, 22, 23) and all cases responded to intravenous diazepam. Thereafter a comprehensive neurologic evaluation including CT scan is indicated. Phenytoin prophylaxis has been reported with continued ECT; however, increased stimulus intensity was required (9). In cases 2 and 3 therapeutic and subtherapeutic phenytoin blood levels raised the seizure threshold above the maximum output of the MECTA and required use of the DALE. Carbamazepine prophylaxis prevented further complications without requiring increased stimulus intensity in cases 1 and 3. In cases 2 and 3, anticonvulsant prophylaxis was discontinued and ECT continued successfully without antidepressants.

We conclude that (a) prolonged seizures and/or status epilepticus may be secondary to an underlying focus and, as such, pre-ECT EEG is indicated; (b) trazodone may lower the seizure threshold; (c) prolonged seizures and/or status epilepticus does not preclude further ECT if clinically indicated; and (d) phenytoin and carbamazepine may have different prophylactic effects with ECT, carbamazepine being the drug of choice.

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Rupture of a Periumbilical Vein Causing Hemoperitoneum in a Cirrhotic Patient

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Abstract

We describe and discuss here the case of a 42-year-old woman with known cirrhosis of the liver in whom we diagnosed hemoperitoneum due to the rupture of a large (4-cm diameter) and distended periumbilical vein.

Eighty-five percent of patients with cirrhosis of the liver eventually develop portal hypertension. The high pressure in the portal system (>20 mmHg) opens up channels between the portal and the systemic venous systems. Some of the major anastomotic channels are the periumbilical veins which pass through the falciform ligament of the liver and join the left branch of the portal vein with the veins of the abdominal wall.

We present this case because it includes two rarities: first, an extremely large periumbilical vein, and second, rupture of that vein causing hemoperitoneum.

Case Report

The patient, a woman aged 42 years, was brought to hospital with abdominal pain, abdominal distention, and shock. She was known to have cirrhosis of the liver, which had been diagnosed 5 years previously by liver biopsy. Since that diagnosis she had been in and out of hospital many times for treatment of common episodes of hepatic encephalopathy. She also had asymptomatic esophageal varices and a radiologic picture of a duodenal ulcer. A few hours before admission she experienced acute abdominal pain, for which she took analgesics, without improvement. She was later found unconscious by her relatives and brought into hospital.

During physical examination the patient was found to be sweating, confused, tachypnoeic, and in shock. Pulse rate was 130/min; systolic blood pressure was 500 mmHg. She also had slightly jaundiced conjunctivae, edema of the ankles, and a prominent caput medusae. During palpation we found the abdomen greatly distended, with slight rigidity in the right hypochondrium and rebound tenderness over the whole of the abdomen. She was pain free during rectal examination; in that examination her physicians felt something spongy in the pouch of Douglas. Blood-test results included hematocrit, 25%; white blood cell count, 5,000 cu mm; urea, 34 mg%, glucose, 186 mg%, potassium, 3 mEq/L. Radiologic examination of the subdiaphragmatic areas was negative.

By nasogastric tube we obtained a bile-stained fluid; we then attempted to counter the shock. Because the patient's pain did not subside and the clinical picture did not change, we performed paracentesis in the right iliac fossa, from which we obtained a blood-stained fluid with hematocrit of 12%. The diagnosis was hemoperitoneum.

Laparatomy was performed by midline incision. Opening the peritoneum abruptly released approximately 4 liters of a blood-stained fluid. A quick inspection of the peritoneal cavity showed the source of the hemorrhage to be a rupture in the wall of a large distended periumbilical vein of the falciform ligament of the liver.

The vein was about 4 cm in diameter (Fig.)

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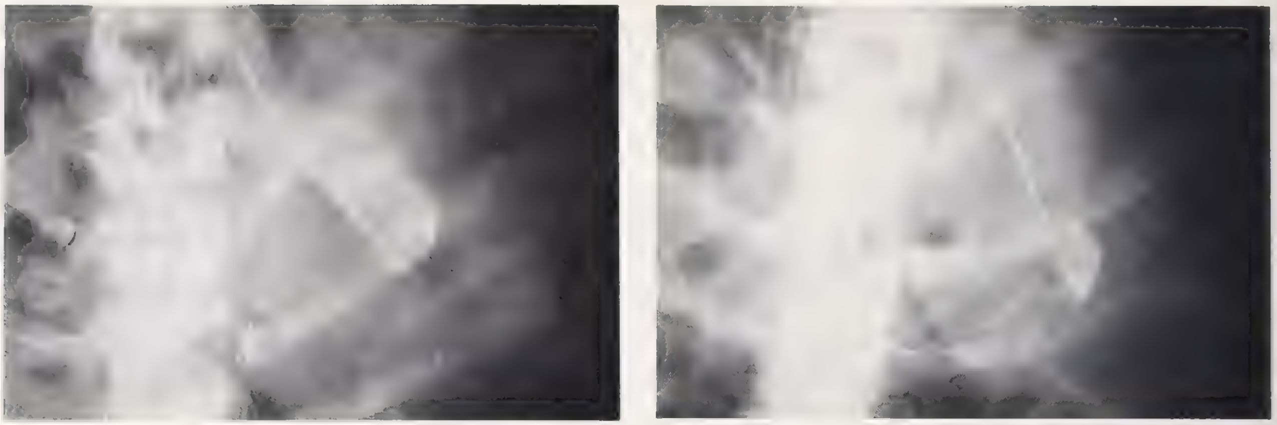

Fig. Venogram showing dilated periumbilical vein.

and resembled a distended gall bladder. It ran from the porta hepatis to the umbilicus. The rupture measured 2–3 mm in diameter and was in the inferior surface in the midsection of the vein. Through this, a jet of venous blood exuded into the peritoneal cavity. The bleeding was stopped immediately by suture of the ruptured portion of the vein. Inspection of the other abdominal organs showed the cirrhosis to be micronodular, typical of Laenek; the spleen was enlarged, but we were unable to find any other enlarged collaterally between the portal and systemic circulation.

The postoperative period was stormy. The patient fell into a hepatic coma, from which she recovered. She finally left the hospital 20 days postoperatively. Eighteen months later the patient was doing well.

Discussion

It is known that patients suffering from portal hypertension are at risk of developing intraperitoneal hemorrhage. The most common cause of intraperitoneal hemorrhage is ruptured hepatocellular carcinoma (1) or carcinoma of the ovary (2); less frequent causes are dissecting aneurysm of the aorta, hepatic adenoma, hemorrhagic pancreatitis, ectopic pregnancy, perforation of a viscus, or tubercular peritonitis.

The perioperative finding of rupture of a large distended periumbilical vein in the patient described here was surprising. Even more surprising was our discovery that no other such cases were described in the literature, except for a tangentially similar case of a ruptured periumbilical varix described by Ross in 1970 (3). A total of 14 other cases of ruptured intraperitoneal varices have been reported (1, 2, 4, 5). It is known that portal hypertension opens the anastomotic channels that exist between the portal and systemic circulation.

Among the major anastomotic channels are the periumbilical veins (6). These veins anastomose the frontal abdominal wall veins with the left branch of the portal vein and follow the path of the round ligament of the liver. Usually there is one major vessel among these veins (Sappey's vein) that runs from the umbilicus behind and superior to or within the surface of the round ligament between the two parts of the falciform ligament ending in the left branch of the portal vein. It is the distention of these veins that causes the characteristic clinical picture of caput medusae. Edwards (7) proposed that, taking into account the large number of anatomic variations of these veins, in any given case the vessel may represent a persistence either of the embryonic right umbilical vein or of segments of the two vitelline veins. A special characteristic of a periumbilical vein is the absence of valves. Anson and McVay (8) claim that in portal hypertension a periumbilical vein may be as large as a little finger in diameter, and that these veins are anastomosed with vein from the gall bladder, the lesser omentum, and the lesser curve of the stomach.

A characteristic finding in patients with cirrhosis and intraperitoneal hemorrhage is, as in this case, pain, abrupt abdominal distention, and hypovolemic shock (2). Paracentesis plays an important role in the diagnosis of intraperitoneal hemorrhage (1, 5). We therefore believe that in patients having these three clinical signs, paracentesis should be performed to exclude intraperitoneal hemorrhage. In hemoperitoneum, the only possible treatment is laparotomy.

We believe that repair of the damaged vessel by suture (or ligation of the varix) is the safest method, taking into account the serious clinical picture for these patients.

A possible question would be whether the performance of a portocaval shunt in these cases would lessen the possibility of rebleeding. However, we were not able to find anything to support this view in the literature.

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Metastatic Choriocarcinoma Masquerading as Septic Pulmonary Embolization

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Abstract

Two weeks after delivering a full-term normal infant, a 20-year-old woman was admitted to the hospital with right hemiparesis and pulmonary nodules on her chest X-ray. Septic pulmonary embolization and mycotic aneurysm were diagnosed on the basis of low-grade fever, heart murmur, and chest radiographic and cerebral angiographic abnormalities. She was treated with vancomycin and chloramphenicol. When the pulmonary nodules did not clear and a right pleural effusion and hilar mass appeared, the patient underwent a thoracotomy, which revealed metastatic choriocarcinoma. Following chemotherapy the chest X-ray abnormalities resolved.

The lung is the most common site of metastatic gestational choriocarcinoma. Pulmonary metastases can have a variety of radiographic appearances. We report the case of a patient in whom choriocarcinoma was incorrectly diagnosed as septic pulmonary embolization.

Case Report

Two weeks after delivering a normal full-term infant a 20-year-old woman was admitted to the hospital with left-sided tonic and clonic movements of the body and right hemiparesis. She had no history of headache, head trauma, or seizure disorder. Two days prior to admission, she was treated for a urinary tract infection with ampicillin. She had no history of intravenous drug abuse, heart murmur, fever, chills, shortness of breath, or pleuritic chest pain.

The patient's temperature was 100°F. An early systolic ejection murmur, grade II/VI, was best heard over the pulmonic area. The lungs were clear. Neurologic examination revealed right hemiparesis.

Hemoglobin was 8.8 gm/dl, hematocrit 25.0%, white blood cell count 27,000/cu mm (80% segmented neutrophils, 12% lymphocytes, 6% basophils, and 2% monocytes). Arterial blood gas studies on room air were pH 7.47, PCO_2 30 mm Hg, PO_2 108 mm Hg.

The chest X-ray showed two nodules, one in the left upper lobe and the other in the left lower lobe, 1.0–2.0 cm in diameter, without well-defined borders (Fig. 1). These nodules were better visualized on chest tomography (Fig. 2). No chest X-ray was taken at the time of delivery.

Cerebral angiography was interpreted as showing a bleeding, mycotic aneurysm of the left middle cerebral artery. Blood cultures were sterile and the echocardiogram did not show vegetations. The patient was treated for six weeks with vancomycin and chloramphenicol for septic pulmonary embolization and a ruptured mycotic aneurysm. Neurologically she improved, but her chest X-ray remained unchanged.

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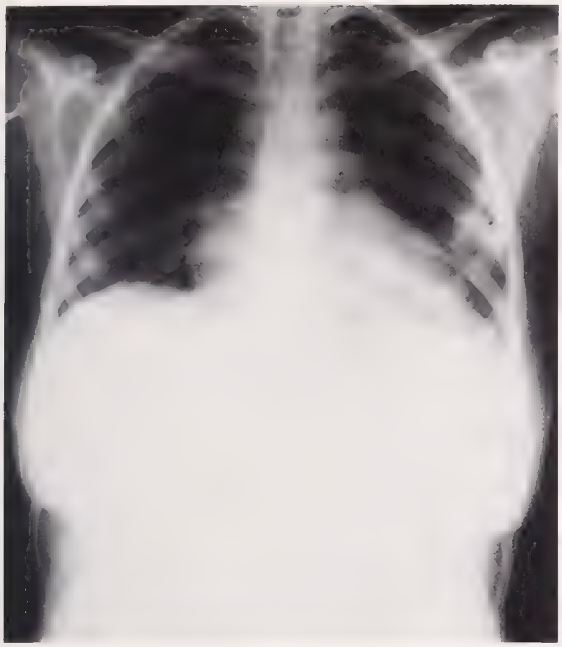

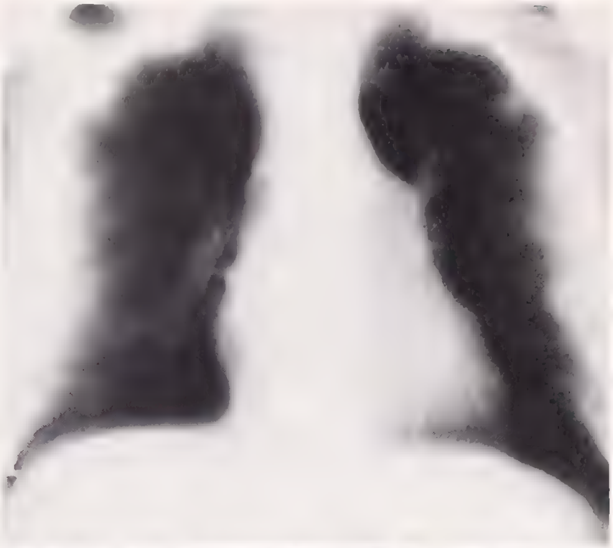

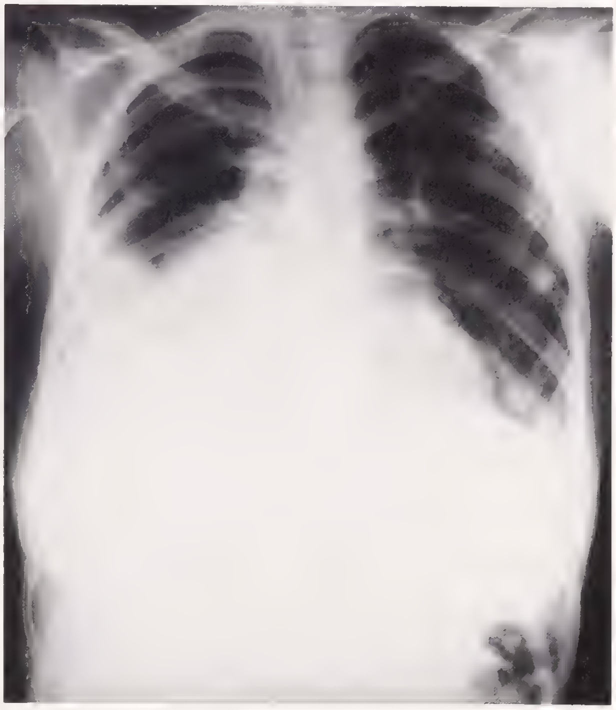

Fig. 1. Upper left Chest X-ray on first hospital admission. Two nodules on left side, one in left upper and one in left lower lung field.

Fig. 2. Upper right Chest tomogram on first hospital admission. Two nodules on left side.

Fig. 3. Right Chest X-ray on second hospital admission, one month later. Right pleural effusion and right hilar mass.

The patient was discharged, and then readmitted to the hospital one month later with right-sided pleuritic chest pain and shortness of breath. She reported no sputum production, hemoptysis, or fever. The chest X-ray revealed a right pleural effusion and large right hilar mass (Fig. 3).

A serosanguineous exudate was aspirated from the right pleural cavity. Since the diagnosis was not clear, the patient underwent a right thoracotomy at which a large hemorrhagic mediastinal mass invading the right horizontal fissure was found. Biopsy of the mass showed metastatic choriocarcinoma.

The level of human chorionic gonadotropin (HCG) was elevated to 290 mIU/ml (HCG was not previously measured). With methotrexate and actinomycin D, the chest X-ray abnormalities resolved and human chorionic gonadotropin levels became zero.

Discussion

Approximately 50% of choriocarcinomas are preceded by hydatidiform moles, 25% by abortions, and 25% by term pregnancies (1). The clinical manifestations of metastatic choriocarcinoma depend on the site of metastases and include hemoptysis, shock from intraabdominal hemorrhage, or a strokelike picture, as in our case (2). The most frequent metastatic site is the lung (1). In a review of chest radiographs of 78 patients with choriocarcinoma and other trophoblastic malignancies, Libshitz et al (3) found 35 patients with pulmonary metastases.

The most common chest radiographic abnormalities in metastases from gestational choriocarcinoma are single or multiple well-defined nodules (3, 4). This type of radiologic picture has been mistaken for lung abscesses and septic pulmonary emboli, as in the case described here (2). Other uncommon abnormalities are ill-defined small nodules (3), pulmonary infarction pattern (4), pleural effusion (3), and hilar mass (3). The roentgenographic picture of the pulmonary infarction pattern is indistinguishable from that due to thromboembolic disease. Pleural fluid is usually hemorrhagic and is due either to pleural involvement by lung lesions or to pulmonary infarction.

The pulmonary metastases of gestational choriocarcinoma may regress and even disappear either spontaneously or after chemotherapy. Rarely, some metastases persist or partially calcify, despite the return of human chorionic gonadotropin levels to normal (5). Swett and Westcott (6) consider residual pulmonary nodules in gestational choriocarcinoma benign if elevated HCG titers return to and remain in the normal range, if other metastases completely resolve, and if residual nodules show no increase in size.

The case we report here emphasizes two points: that the diagnosis of metastatic choriocarcinoma should be considered in all women in the childbearing years who have unexplained abnormal chest radiographic findings, and that choriocarcinoma may masquerade as septic pulmonary embolization. With early detection and treatment, the prognosis in choriocarcinoma is excellent.

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Concurrent and Noncontiguous Squamous and Transitional Cell Carcinomas of a Bladder

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Abstract

A case is presented in which the involvement of the urinary bladder by papillary transitional cell carcinomas was associated with a pure, pearl-forming squamous cell carcinoma. Mapping of the urinary bladder showed that the squamous cell carcinoma was noncontiguous with the transitional cell carcinomas and that the squamous metaplasia, with and without atypia, and atypical urothelium were irregularly distributed in the bladder. The findings suggest a pathogenetic kinship between these lesions.

Squamous cell carcinoma of the bladder is relatively infrequent in the West but it is prevalent in areas of the world infested with *Schistosoma hematobium* (1–3). Papillary transitional tumors of the bladder comprise the majority of all primary bladder neoplasms. Although the transitional cell carcinoma may have areas of squamous differentiation, its association with a separate and pure squamous cell carcinoma in the bladder has not hitherto been described in the literature. This report describes the result of mapping of a bladder which showed such an association.

Case Report. A 94-year-old white man was evaluated for intermittent hematuria in November 1980. Cystoscopy revealed multiple papillary tumors interspersed on the bladder mucosa, a large papillary tumor occupying the major portion of the dome, and a polypoid broad-based tumor in the trigone. Partial resection of the tumors was attempted suprapubically and via urethra in November 1980 and in August and September 1981. The morphologic configuration of the submitted specimens was consistent with a grade II papillary transitional carcinoma and a squamous cell carcinoma. The former was interpreted as being a tumor separate from the latter, which superficially invaded the muscle layer of bladder wall.

The patient was readmitted in February 1982 for intermittent bleeding of an ulcerating submandibular lesion. Biopsy of the lesion showed a poorly differentiated squamous cell carcinoma infiltrating the reticular dermis. The patient received 4500 rads to the left lateral cheek field in March 1982 and 2400 rads in December 1982 to the enlarged neck lymph nodes. The patient did not receive radiotherapy for the bladder tumors throughout the course of his disease and died in June 1983 after a short febrile episode.

The patient was born in Russia, came to the United States as a youngster, and lived his entire life in New York City. He smoked two to three cigarettes per day and drank on a social basis. The patient had no known history of exposure to chemical carcinogens.

Autopsy Report. The autopsy revealed a necrotizing ulcer in the left mandibular region and en-

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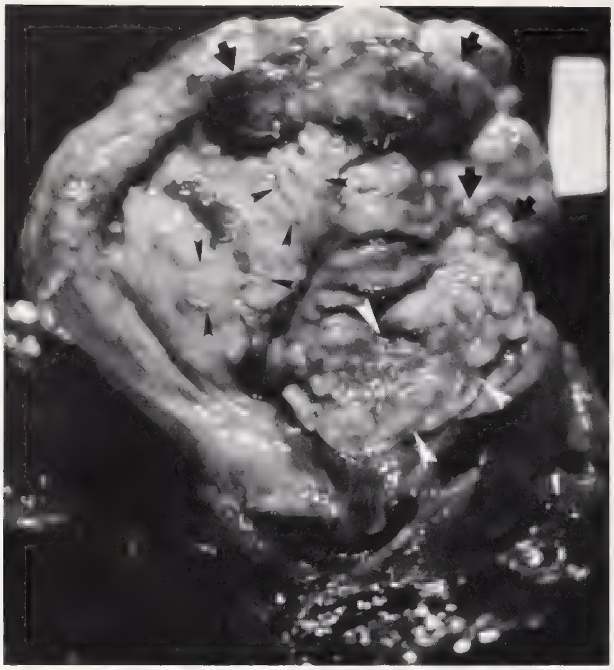

Fig. 1. Left Bladder showing multiple papillary tumors (black arrows), squamous cell carcinoma (white arrowheads) and some areas of squamous metaplasia (black arrowheads).

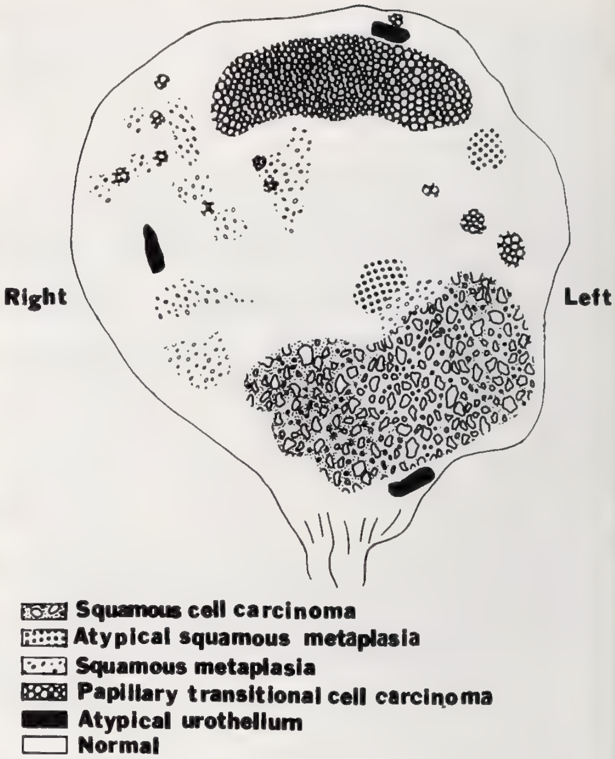

Fig. 2. Right Mapping of bladder showing distribution of papillary tumors, squamous cell carcinoma, areas of squamous metaplasia, with and without atypia, atypical urothelium, as well as normal mucosa.

larged and indurated supraclavicular lymph nodes. There were several small bilateral subpleural nodules. Examination of the bladder showed a large, 4.5-cm-diameter tumor and several papillary tumors in the dome 1 cm or less. Smaller tumors could also be observed on the posterior wall. A large, broad-based, solid tumor extended from the trigone to the left lateral wall and invaded beyond the confines of the urothelium. Several white plaques were irregularly distributed in the bladder mucosa (Figs. 1, 2). The other organs did not show significant pathology.

Microscopic examination of the submandibular ulcer showed a solidly growing carcinoma composed of cells in which only occasional keratinization of individual cells could be observed. Moreover, only scattered intercellular bridges were seen. The tumor was defined as a poorly differentiated squamous cell carcinoma (Fig. 3). The tumor infiltrated deeply into the subcutaneous tissues. The neoplasia effacing the architecture of the neck lymph nodes and the subpleural nodules exhibited a similar morphology to the submandibular neoplasia.

Mapping of the bladder. The entire bladder urothelium was mapped, as previously described (4–6). Briefly, the bladder was fixed in buffered formalin and the entire bladder was carefully blocked. Individual blocks, 2 cm in length and 0.5 cm in width, were appropriately identified and embedded in paraffin, and were submitted for routine sectioning. A total of 60 slides was studied. A diagrammatic representation of the appearance of entire bladder epithelium was drawn on the basis of microscopic examination of the slides. The lesions were defined according to published criteria (7).

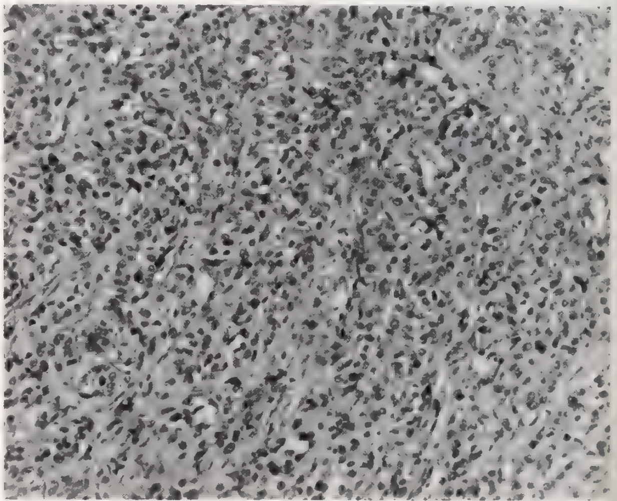

Fig. 3. Poorly differentiated squamous cell carcinoma of submandibular lesion.

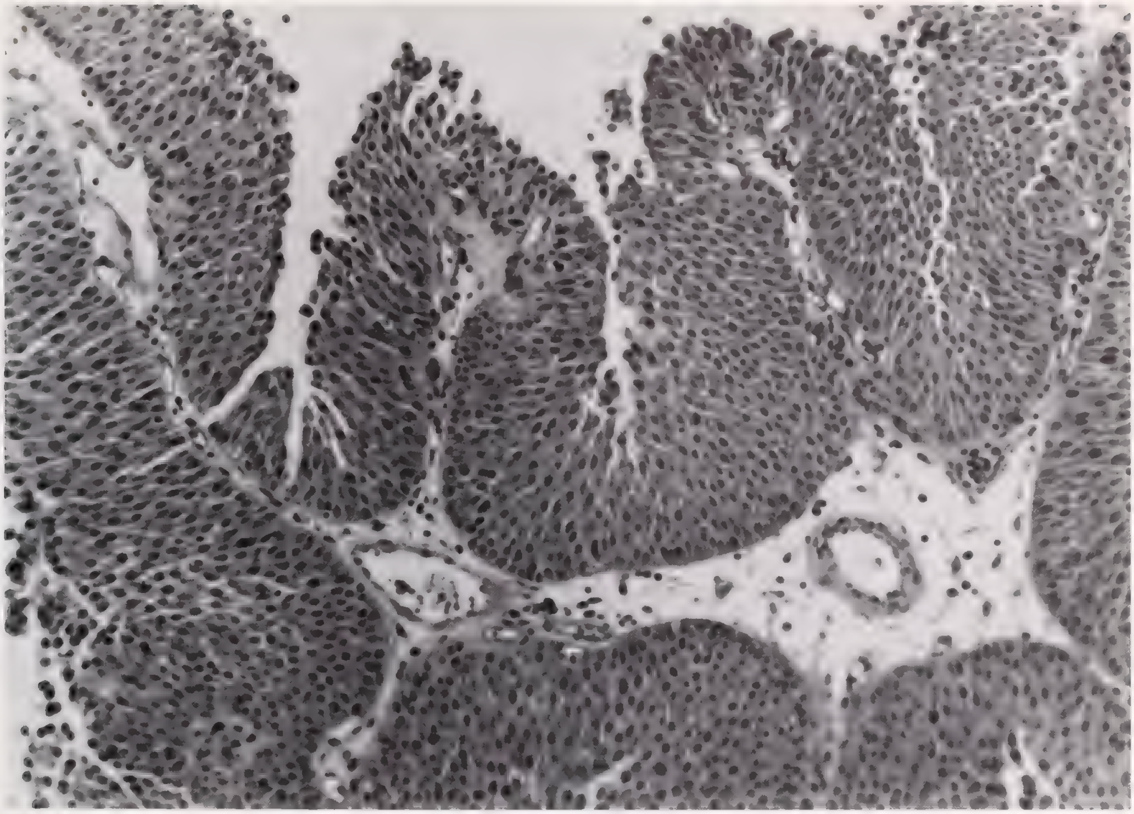

Fig. 4. Papillary transitional cell carcinoma, grade II, in dome of bladder.

The large lesion in the dome (Figs. 1, 2) had papillae lined by hyperplastic urothelium (Fig. 4). Some nuclear abnormality, mainly enlargement and hyperchromasia, and focal squamous and glandular metaplasia were present. This tumor was classified as a grade II papillary transitional cell carcinoma. The morphology of the tumor resembled that seen in the original biopsy. The tumor had a pushy border; however, it did not extend beyond the confines of the urothelium.

The small lesions in the dome and posterior wall of the bladder had papillary structures lined by a hyperplastic urothelium, either without significant or with some nuclear abnormalities, classified respectively as grade I or II papillary transitional cell carcinomas. These tumors did not invade lamina propria or muscle layer of the bladder wall.

The solid tumor in the trigone was characterized by the presence of keratinized single cells or sheets of cells and concentric aggregates of keratinized cells, the so-called squamous pearls. Squamous cells showed mild to moderate atypia. This tumor did not have any area with features of transitional cell carcinoma and was classed and graded as a pure squamous cell carcinoma, grade I. It invaded the lamina propria and muscle layer of the bladder wall (Fig. 5). Its morphology did not differ from that seen in the original biopsy. Areas which were grossly identified as white plaques were composed of mature and nonkeratinizing squamous epithelium with variable amount of glycogen. This was designated as squamous metaplasia.

In proximity to the large transitional and squamous cell carcinomas, surface epithelia with recognizable squamous features, such as intercellular bridges and intracytoplasmic keratin, exhibited nuclear pleomorphism, hyperchromasia and increased nuclear cytoplasmic ratio through its full thickness. These epithelia had both flat and papillary features and were designated as atypical squamous metaplasia to squamous carcinoma in situ (Fig. 6).

The intervening epithelium between the lesions was composed of either normal urothelium with less than seven layers of cells and an innermost layer of umbrella cells or atypical urothelium with less orderly arrangement of cells and nuclear abnormalities.

Discussion. This case portrays a multifocal and diverse morphologic expression of an unresting and abnormal bladder epithelium which ranged from mildly atypical urothelium, squamous

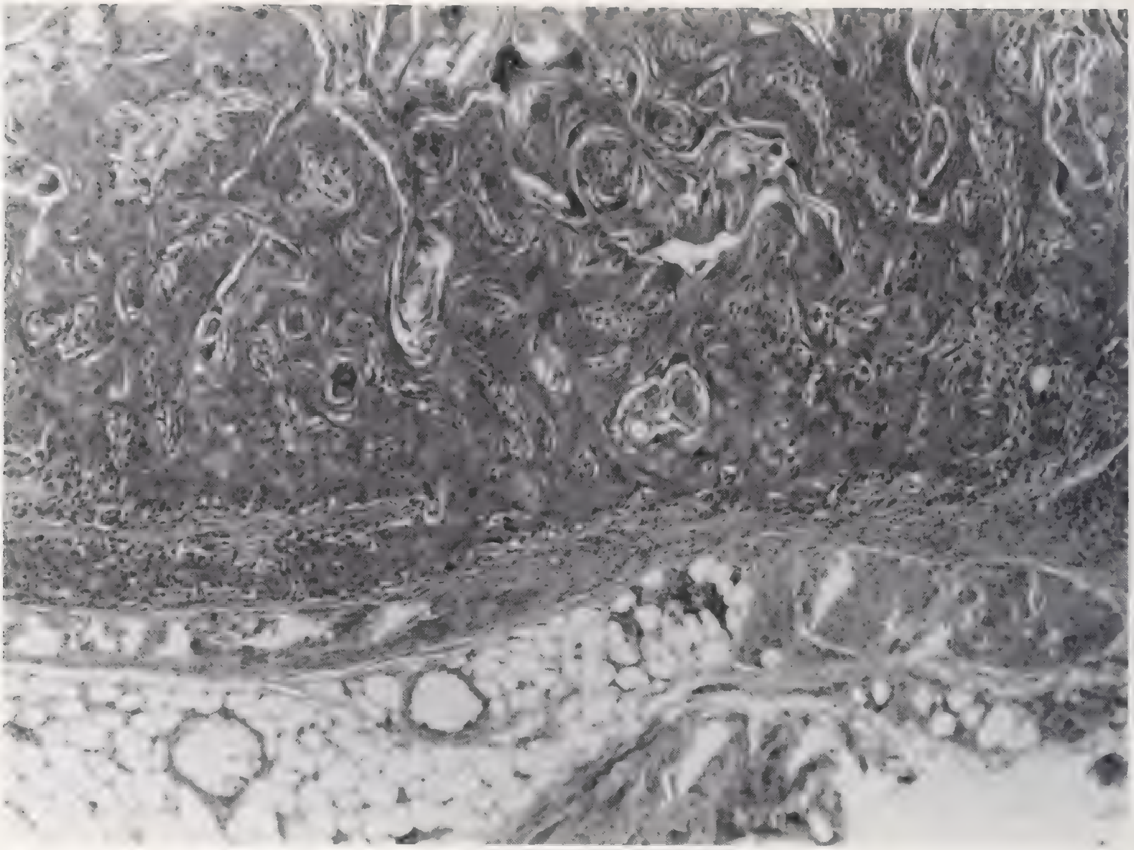

Fig. 5. Squamous cell carcinoma, grade I, in trigone of bladder, invading muscle layer of bladder wall and approaching perivesical fat.

metaplasia with and without atypia, to multicentric foci of papillary transitional carcinomas and a single squamous cell carcinoma. Transitional cell carcinomas commonly harbor areas of squamous or glandular elements. However, the occurrence of a pure, separate, and morphologically different tumor in association with transitional cell carcinoma is a rare event. To our knowledge, there has been no prior report illustrating the concurrence of a noncontiguous squamous and transitional cell carcinoma in the bladder. Mapping of the urinary bladder in the present case demonstrated such an event.

The squamous metaplasia with and without atypia in this case comprised an epithelial abnormality interspersed in the urothelium of bladder. The relationship of squamous metaplasia to squamous cell carcinoma has been a major area of controversy. Some subscribe to the view that squamous metaplasia may be precancerous (8–11). Experimentally induced, invasive squamous cell carcinomas commonly develop from flat epithelial changes akin to atypical squamous metaplasia (12). Similarly, in humans, invasive squamous cell carcinomas of the bladder most likely arise from squamous carcinoma in situ (13). Atypical squamous metaplasia approaching carcinoma in situ was found in the present case in an area adjacent to the invasive squamous carcinoma.

The submandibular neoplasia, with its poor differentiation, seems to represent a primary tumor separate from the squamous cell carcinoma of the bladder. Tumors involving the neck lymph nodes and the lung had morphologic features similar to the submandibular lesion, and it is likely that they represent metastatic spread from the latter and that they did not originate from keratinizing squamous cell carcinoma within the bladder. The likelihood that the keratinizing squamous cell carcinoma of bladder also represents a metastasis from the submandibular tumor is a remote possibility due to marked difference in sequence of their development and contrast in their morphology and degree of differentiation. The presence of squamous carcinoma in situ in the bladder, which is the lesion preceding the invasive carcinoma, supports the hypothesis that the squamous carcinoma of the bladder was a separate primary tumor.

It is a dilemma whether metachronously or synchronously developed lesions of the bladder in

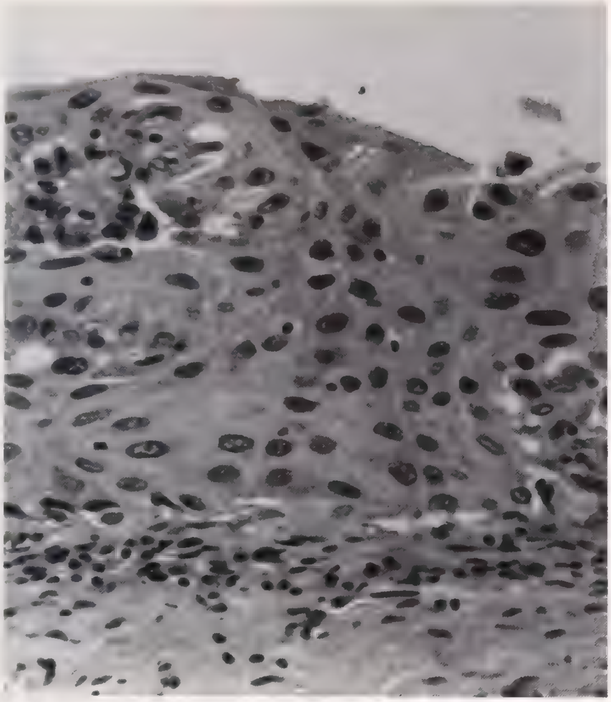

Fig. 6. Squamous metaplasia with marked atypia.

the present case reflect the potential of the bladder epithelium to express diverse morphologic changes to various environmental influences, or merely exemplify focal expressions to a single stimulus. However, the latter is more likely, since the presence of atypical squamous metaplasia in the vicinity of, and squamous metaplasia within, the large transitional carcinoma may indicate pathogenetic kinship between these lesions. The patient did not originate from those parts of the world in which infestation with *Schistosoma hematobium* is common. As far as could be ascertained, the patient was not exposed to any chemical carcinogen. The patient had a history of cigarette smoking; however, the descriptive epidemiology of bladder cancer was recently shown to differ from the expected pattern of an occupationally linked disease or cigarette smoking (14).

The present case illustrates that under favorable conditions morphologically similar or dissimilar neoplastic lesions may occur in the urothelium.

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Ureteral Herniation: Case Report and Literature Review

Jay D. Burstein, M.D., Sam Lipshitz, M.D., Robert J. Weinstein, M.D., Paul Boulinakis, M.D., L. Seidman, M.D., and Allan Fredland, M.D.

Abstract

Herniation of the ureter is an infrequent event. A case report is presented and the literature reviewed. Preoperative recognition of this disorder will ensure proper treatment.

Herniation of the ureter is an infrequent event, as demonstrated by 127 reports in the literature (1–3). Although the condition was first recognized during an operative procedure by Reichel in 1892, only in 1937 was a urographic preoperative diagnosis made, by Dourmashkin (4, 5). Since then, the majority of cases have remained undiagnosed until the time of surgery. A case report and a review of this subject are presented here to facilitate preoperative recognition, which can be essential in proper management.

Case Report. The urology service was consulted to evaluate a 48-year-old obese, diabetic, hypertensive white man with urinary incontinence and scrotal mass. The patient reported that he had no scrotal pain or discomfort and that the enlargement had been present for several years. Further questioning revealed a weak urinary stream with incontinence suggestive of the overflow type. There was no other urologic complaint. Significant events in his history included splenectomy performed for hypersplenism and repair of a strangulated umbilical hernia performed one week prior to our consultation.

Physical examination disclosed a markedly obese man in no distress. The abdomen was soft, with incisional tenderness about a well-healing midline incision. No masses were appreciated. The left hemiscrotum contained a 6 × 10 cm, nontender, ballotable, soft mass which partially transilluminated, but was nonreducible. Both testes were palpable and of normal size and consistency.

Hemoglobin was 11.2% and hematocrit 34%. White blood count was 1,200 with lymphocytosis. BUN was 18, creatinine 1.8, and blood glucose 108. Urine culture yielded no growth. Excretory urography revealed delayed function and gross hydroureteronephrosis of the left kidney. Urodynamic and cystoscopic findings were consistent with diabetic uropathy. Left retrograde pyelography disclosed herniation of the lower ureter with moderate hydroureter of the distal segment (Fig. 1).

Exploration, performed through a Gibson incision, revealed a large sliding hernia, the ureter involving the lateral wall. Omentum was contained in the peritoneal sac (Fig. 2). Resection of redundant ureter, ureteroureterostomy, and hernia repair were then performed. A 5 French ureteral catheter was used as a stent. Convalescence was unremarkable; the stent was removed on the eighth postoperative day. BUN and creatinine remained unchanged.

Discussion. Most ureteral hernias are either inguinal, femoral, or sciatic (1). They are commonly of the inguinal type, occurring twice as often as

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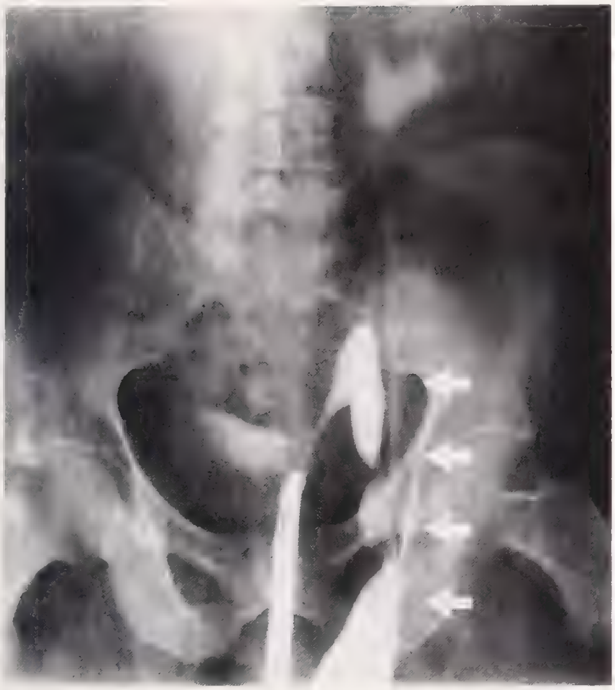

Fig. 1. Left Left retrograde pyelogram. Ureteroinguinal hernia.

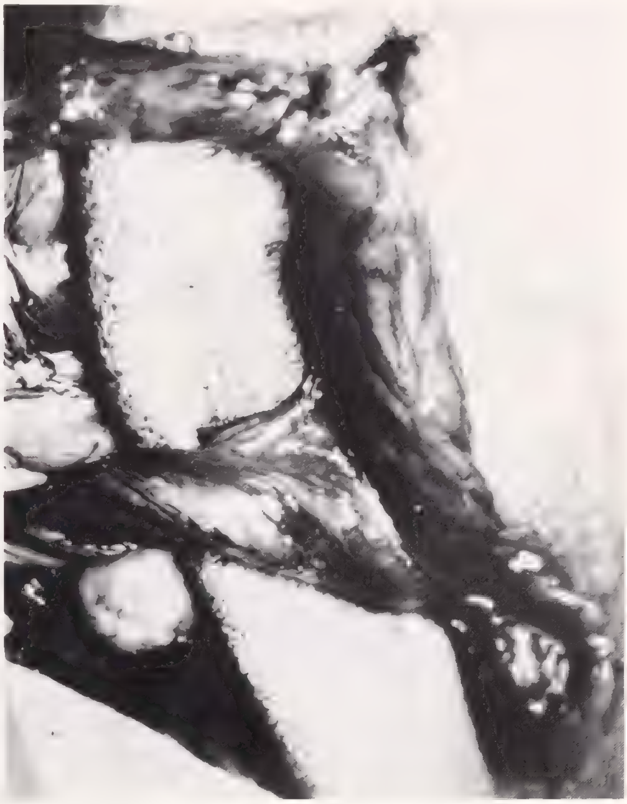

Fig. 2. Right Intraoperative specimen demonstrating herniated portion of ureter.

femoral hernias. The highest incidence is between the fourth and sixth decades. Reports have confirmed herniation into the chest through the foramen of Bochdalek (6) and into the space between the psoas muscle and iliac vessels (7).

Two types of ureteroinguinal hernias occur:

The paraperitoneal variety has a true peritoneal sac, the ureter forming part of its wall, thus forming a sliding hernia. Pathogenesis is by adherence of the ureter to the posterior peritoneum associated with traction exerted by the sac as it descends through inguinal structures.

The extraperitoneal type has no hernial sac and is approximately one-fifth as common (1). These are considered congenital in origin, resulting from anomalous development of the ureteric bud from the wolffian duct and traction during testicular descent (7). Associated herniation of the bladder may occur with either type but is more common with the extraperitoneal form.

Ureterofemoral hernias, which are situated medial to the femoral vein, usually occur in females, as do femoral hernias in general. Usually located on the right side, they are almost invariably accompanied by a sac. This is considered an acquired hernia produced by traction of the peritoneum, to which the ureter adheres (1).

Ureterosciatic hernias typically herniate through the superior compartment of the greater sciatic foramen, a space bounded inferiorly by the piriformis muscle and by the ilium superiorly. This variety affects adult women, and children of both sexes, apparently secondary to piriformis atrophy associated with hip and neuromuscular disorders (8).

The symptoms of ureteral herniation are obstruction and hydronephrosis. However, most such herniations are asymptomatic. If the bladder accompanies the ureter, irritative symptoms are common. Occasionally, a massively dilated ureter acting as a conduit results in double voiding.

Radiologic diagnosis is simple when the ureter is seen in an extrapelvic location. However, excretory urography may not provide sufficient visualization because of the small amount of contrast that reaches the herniated portion. Retrograde pyelography typically demonstrates a "curlique ureter" (9), a spiral "loop-the-loop" produced by superimposition of the afferent and efferent limbs as they cross the hernial hiatus (1). The loop axis is horizontal in sciatic and vertical in inguinal and femoral types.

The surgical approach consists of resection of redundant ureter, reanastomosis, and herniorrhaphy. Care must be taken to identify pertinent structures prior to complete dissection. If provocation of ureteral peristalsis or fine-needle aspiration fails, intravenous injection of methylene blue may be beneficial. With associated bladder involvement, avoidance of the vas is of fundamental importance.

In our case of paraperitoneal ureteroinguinal hernia, the preoperative diagnosis led to an uneventful operative course. To avoid potential complications of inadvertent lower urinary tract injury, we agree with Barquin and Madsen (10) that patients with large hernias should undergo urographic investigation.

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Inclusion Body Myositis: Case Reports and a Reappraisal of an Underrecognized Type of Inflammatory Myopathy

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Abstract

Chronic progressive weakness and wasting of the proximal and distal muscles in two elderly patients is reported. Both patients had clinical and laboratory findings suggestive of active inflammatory myopathy, but the stigmata of collagen vascular disease were lacking, and a search for malignancy was negative. Light microscopy of their muscle biopsies showed findings consistent with inflammatory myopathy, but in addition, numerous cytoplasmic vacuoles lined with basophilic granules were found. Conglomerations of cytomembranous whorls and abnormal cytoplasmic filaments were observed in myofibers by electron microscopy. This type of inflammatory myopathy, termed "inclusion body myositis," deserves recognition because it is distinct from the other types clinically and microscopically and is resistant to immunosuppression.

Polymyositis, as conventionally defined, is an acquired inflammatory myopathy characterized clinically by symmetric weakness of proximal muscles. When skin rash accompanies this muscle weakness, the term "dermatomyositis" is applied. The serum "muscle enzymes," particularly creatine kinase, are frequently elevated. The usual electromyography findings include signs of muscle membrane irritability (positive sharp waves, fibrillation potentials, and high-frequency motor unit discharges), short-duration, small-amplitude, easily recruitable polyphasic motor units, and normal motor and sensory nerve conduction velocities. Histologically, the hallmarks of the disease include muscle necrosis, phagocytosis, and regeneration as well as mononuclear infiltrates and, sometimes, perifascicular atrophy. When all these features are present, the diagnosis of polymyositis or dermatomyositis can be made.

Recently, we investigated a man and a woman who were thought initially to have idiopathic polymyositis and whose cases merit further description because of the slow, protracted course of their disease, resistance to immunosuppressive treatment, and unusual muscle biopsy findings. These cases, we believe, were "inclusion body myositis," a distinct but underrecognized type of myopathy that is still being diagnosed and treated as idiopathic polymyositis with inordinate frequency.

Case Reports

Patient 1. Symmetric progressive weakness of the lower extremity muscles developed insidiously in a 79-year-old woman, twenty-five years prior to neurologic evaluation. Getting up from a chair, climbing stairs, and turning in bed had become progressively more difficult. A few years after onset of lower-limb weakness, she

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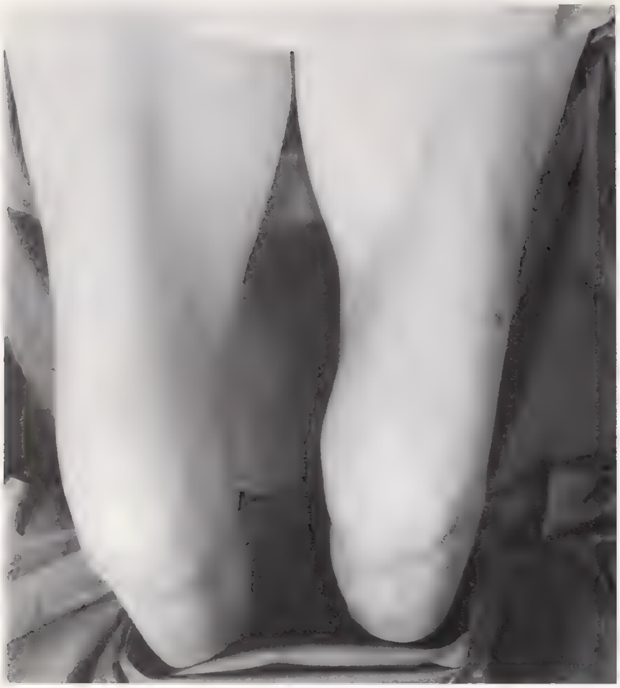


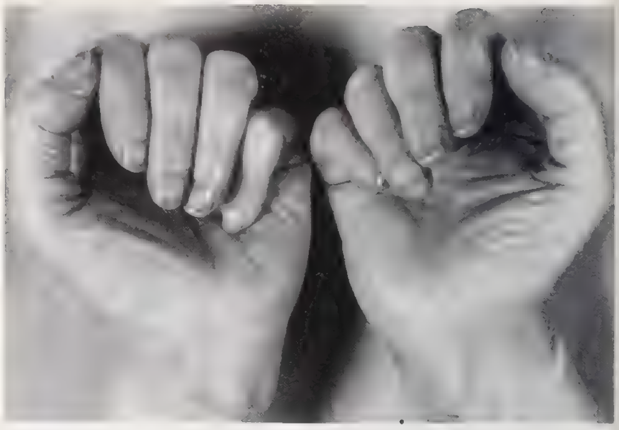


Fig. 1. Left Patient 1. Marked atrophy of quadriceps femoris muscle.

Fig. 2. Right Patient 1. Patient is unable to make tight grip.

found it difficult to turn door knobs, hold a pencil, or open cans. She also complained of dysphagia, especially to soft foods. She experienced no diurnal fluctuation in her strength and no obvious cutaneous symptom, myalgia, or arthralgia. An elevated serum creatine kinase of 500 U/L (normal, up to 140), elevated erythrocyte sedimentation rate, abnormal electromyographic findings, and abnormal quadriceps femoris muscle biopsy (formalin-fixed, paraffin sections) led to the diagnosis of polymyositis. Search for malignancy was negative. Response to high-dose prednisone (60–100 mg/day) at first seemed dramatic, but in subsequent years her weakness progressed until she was confined to a wheelchair, despite her continued use of prednisone. Her medical and family history were otherwise not relevant.

General physical examination revealed a soft systolic murmur at the apex and posterior subcapsular cataract bilaterally. Joint deformities, swelling, and cutaneous abnormalities were absent. On neurologic examination she was unable to stand or walk and had diffuse, flaccid muscle weakness and atrophy, particularly of the quadriceps femoris muscle (Fig. 1). She had mild bilateral facial weakness, and her handgrip was very weak bilaterally (Fig. 2). Muscle strength testing (Table 1) revealed her muscle stretch reflexes to be hypoactive in the upper extremities and ankles but present at the knees despite atrophic quadriceps femoris. There were no fasciculations.

Findings were normal on routine hemogram, urinalysis including myoglobin screen, ESR, blood chemistries, serum protein and immunoglobulin electrophoresis, serum complements, thyroid function, antinuclear antibody, lupus erythematosus cell preparation, rheumatoid arthritis factor, and serum Venereal Disease Research Laboratory test. Serum creatine kinase, lactic dehydrogenase, and alkaline phosphatase were mildly elevated.

Osteoporotic bony changes were noted in the thoracic spine radiographs. Delayed emptying and hypoperistalsis of the esophagus were noted by barium swallow. Chest and cervical spine radiographs and electrocardiogram were normal.

Electromyogram of the tibialis anterior, gas-

TABLE 1

Muscle Strength Testing, Medical Research Council Scale

	Patient 1		Patient 2	
Muscle	Right	Left	Right	Left
Deltoids	4/5	4/5	4/5	4/5
Biceps brachii	4/5	4/5	4/5	4/5
Triceps	3/5	3/5	4/5	4/5
Wrist extensors	3/5	3/5	4/5	4/5
Wrist flexors	3/5	3/5	4/5	4/5
Hand grip	Weak	Weak	Weak	Weak
Neck flexors	4/5		4/5	
Neck extensors	5/5		5/5	
Iliopsoas	4/5	4/5	4/5	4/5
Hamstrings	3/5	3/5	4/5	4/5
Quadriceps femoris	2/5	2/5	1/5	1/5
Ankle dorsiflexors	5/5	5/5	4/5	4/5
Ankle plantar flexors	5/5	5/5	5/5	5/5

trocnemius, vastus lateralis, deltoids, and first dorsal interosseous muscles showed positive sharp waves and fibrillation potentials and a mixture of normal and small-amplitude, normal-duration, easily recruitable motor units. The nerve conduction velocities (median, common peroneal, and sural nerves) and a supramaximal repetitive nerve stimulation study were normal.

Despite combined treatment with azathioprine, 100 mg daily, and prednisone, 80 mg daily, no significant improvement in her strength was noted.

Patient 2. A 65-year-old man began to experience difficulty getting up from a chair and toilet seat five years prior to neurologic evaluation. A year later he noted difficulty raising his arms and inability to make a tight grip. He never complained of dysphagia, myalgia, arthralgia, or cutaneous symptoms. His muscle weakness progressed until he had difficulty walking without falling, and his muscle bulk, particularly in the thighs, diminished considerably.

The only relevant data in his medical and family history were diabetes mellitus and hypertension, diagnosed in another hospital two years after onset of his muscle weakness.

Examination showed diffuse muscle wasting without fasciculations and weakness of the limb-girdle musculatures. In muscle strength testing (Table 1), he could barely flex the left middle and ring fingers. His knees were locked in hyperextended position when he stood, and he had a prominent steppage gait. The muscle stretch reflexes were preserved, but were absent at the knees.

Except for mildly elevated serum creatine kinase, the results of an extensive laboratory workup, particularly a search for collagen vascular disease and malignancy, were negative.

Electromyogram of the biceps brachii, deltoids, first dorsal interosseous, and iliopsoas muscles showed widespread positive sharp waves, fibrillation potentials, and abundant short-duration, small-amplitude polyphasic motor units. His nerve conduction velocities (median, ulnar, common peroneal, and posterior tibial) were normal.

The patient refused trials of steroids, immunosuppressive therapy, and plasmapheresis.

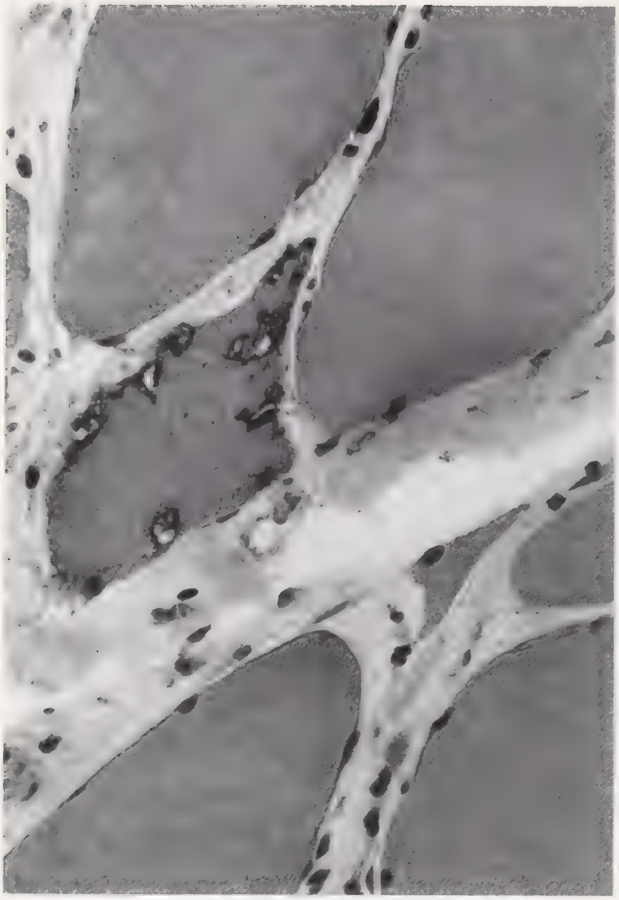
Materials and Methods

In both patients the muscle biopsies were taken via the open method under local anesthesia. The left rectus femoris and left deltoid were sampled in Patient 1 and the left biceps brachii in Patient 2. From each muscle biopsy, one piece was quick-frozen in liquid nitrogen and processed for cryostat sections, direct immunofluorescence microscopy, and a battery of histochemical stains using the technique described by Dubowitz and Brooke (1). The second piece was fixed in formalin and processed for paraffin sections. The third piece was processed for electron microscopy.

The light microscopy of the muscle biopsies of 17 patients (aged 15–77) bearing the diagnosis of idiopathic inflammatory myopathy (polymyositis, 14; dermatomyositis, 2; eosinophilic polymyositis 1), and examined in our laboratory during 1977–1983, were reviewed and compared with the materials. In addition, for comparison we reviewed biopsy preparation from 2 patients with myofasciitis, and 20 patients with various neuromuscular disorders (Duchenne muscular dystrophy, spinal muscular atrophy, idiopathic muscle aches and cramps, facioscapulohumeral dystrophy, amyotrophic lateral sclerosis, myotonic dystrophy, mitochondrial myopathy, nemaline myopathy, and oculopharyngeal dystrophy).

Results

Cryostat sections, hematoxylin-eosin stained, from both patients showed fibers with numerous vacuoles lined with basophilic granules (Fig. 3). These vacuoles were not seen in the paraffin sections. They did not show acid phosphatase activity. Some rimmed vacuoles contained eosinophilic material. There was a spectrum of fiber size in the biopsy specimens, and scattered, rounded, and angular atrophic fibers were noted. Group atrophy was seen in some areas. Both hypertrophic and atrophic fibers contained vacuoles. Occasional dark-staining fibers were seen in the trichrome stain and NADH-tetrazolium reductase reaction. Degenerating fibers and mononuclear cellular infiltrates of varying density (Fig. 4) were noted. There was also evidence of partial invasion of occasional nonnecrotic fibers by mononuclear cells. A few regenerating fibers were recognized by their purplish cytoplasm and vesicular nuclei. Central nuclei were present in about 10% of fibers, and some of these nuclei were unusually large and had peripherally displaced chromatin; other nuclei were small, pyknotic, and clumped. Endomysial connective tissue was mildly to moderately increased. The number of capillary lumina was not assessed, although subjectively the number appeared to be increased. Neither type grouping nor selective fiber-type atrophy was seen in adeno-



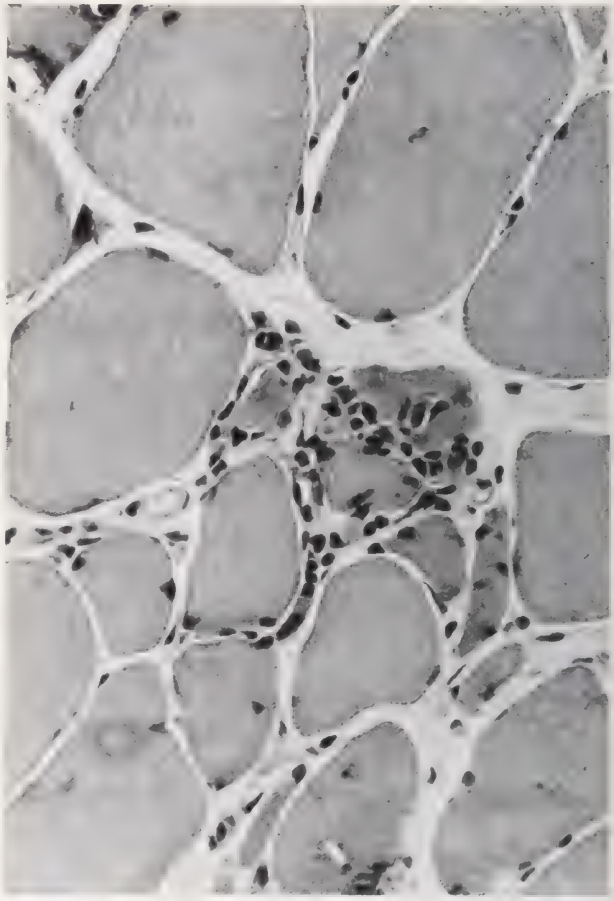


Fig. 3. Left Patient 2. Transverse section of biceps brachii muscle showing fiber with vacuoles filled and lined with basophilic granules (H&E, original magnification ×500).

Fig. 4. Right Patient 1. Transverse section of deltoid muscle showing mononuclear cellular infiltrates with partial invasion of muscle fibers. Vacuoles are also present in some fibers (H&E, original magnification ×500).

sine triphosphatase reactions. Both type 1 and type 2 fibers contained vacuoles. No specific abnormalities were encountered in oil red-O, adenylate deaminase, phosphorylase, and PAS stains. The results of direct immunofluorescence microscopy (anti-IgG, anti-IgM, anti-C3, anti-C4, and antifibrinogen) were negative.

Cryostat sections of biopsy specimens from patients with various inflammatory myopathies and other neuromuscular disorders did not show basophilic-lined vacuoles or unusually large nuclei, except for an isolated fiber containing a slitlike sarcoplasmic vacuole lined by a thin basophilic rim, taken from a patient with oculopharyngeal dystrophy.

The most frequent and most prominent ultrastructural finding consisted of accumulations of electron-dense membranous whorls, latticelike "honeycomb" structures, and dense bodies (some of which are large and similar in appearance to lipofuscin). These accumulations are believed to represent the basophilic granules seen by light microscopy. Some accumulations were subsarcolemmal in location, but more were found within muscle fibers. Most of the cytoplasmic structures surrounding these accumulations appeared normal. Some leaflets of whorls displayed periodicity of approximately 4 nm. Glycogenlike granules of 25-nm mean diameter were often found in the vicinity of membranous whorls and also within the cytomembranous loops.

Circular accumulations of whorled membranes surrounded abnormal cytoplasmic masses made up of small membrane-delimited vacuoles and abnormal filaments (Fig. 5); amorphous "groundglass" material surrounded at least in part by limiting membrane; vacuoles of small and medium size interspersed with granules appearing to be glycogen, although somewhat less electron; dense than usual; disorganized masses of thick and thin myofilaments with smeared Z disc material; and large empty or nearly empty vacuoles. The abnormal filaments measured 18 nm as in mean diameter (range, 14–20 nm). Although similar in size to thick myofilaments, at high resolution, the abnormal filaments ap-

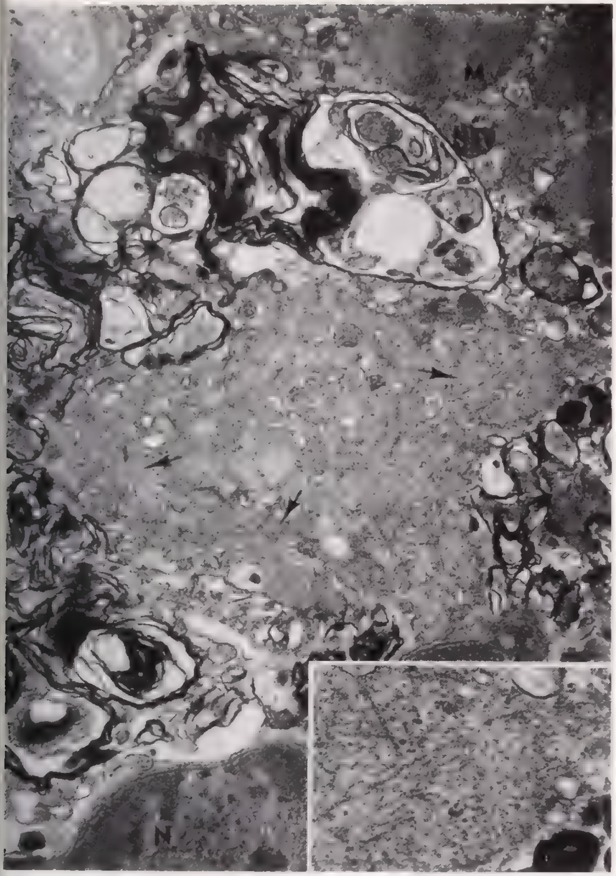


Fig. 5. Patient 2. Electromyogram of inclusion body containing filaments (arrows). Surrounding inclusion are cytomembranous whorls containing glycogenlike granules. Normal muscle fibers (M) at upper right and normal nucleus (N) below (original magnification ×19,950). Insert of filaments at lower right (original magnification ×42,000).

peared more tubular in structure and more undulating along longitudinal contour as compared to thick myofilaments. Also, the abnormal filaments were not associated with thin myofilaments. Infrequently found were degenerating fibers with electron-dense cytoplasm and condensed, disorganized myofilaments, and fibers with much glycogen throughout the cytoplasm but normal-appearing groups of thick and thin myofilaments. Occasional nuclei showed increased infolding of nuclear membranes and excess heterochromatin. No abnormal filaments were identified within nuclei or cytoplasmic invaginations into the nuclei ("pseudoinclusions"). Accumulations of mitochondria without paracrystalline inclusions were encountered in subsarcolemmal and more central locations.

Discussion

The heterogeneity of polymyositis has been the subject of recent critical reviews (2, 3). Because of its diverse etiologies and association with various disorders, polymyositis can reasonably be considered a syndrome and not a disease. Unless homogeneity of patient samples is established, the evaluation of therapy for the condition is likely to be unsatisfactory. Unfortunately, criteria set forth to establish the diagnois are beset with flaws. These criteria merely suggest the inflammatory nature of the muscle disease, but have no etiologic basis, and the mere presence of cellular infiltration does not make a muscle disease inflammatory. In the absence of inflammation, it is difficult to distinguish the condition from various sporadic storage myopathies such as carnitine deficiency, adult-onset acid maltase deficiency, and other glycogen storage myopathies, and from sporadic limb-girdle "dystrophies."

In recent years, the human inflammatory myopathies have been classified conveniently by several authors (3–5). Those included in the idiopathic group comprise the largest number, among which are idiopathic polymyositis, dermatomyositis (adult and juvenile), the "poly" or "dermato" form complicating collagen vascular diseases and malignant neoplasms, and a miscellaneous group. Included in the miscellaneous group are eosinophilic polymyositis (6), familial forms with restricted distribution (7, 8), sarcoid myopathy (9), giant-cell polymyositis with thymoma (10), and other less common forms associated with various conditions (11, 12).

A distinct but infrequently recognized type has emerged in recent years. The demonstration of viruslike particles in muscle fiber nuclei and cytoplasm by Chou in 1967 (13), and by Yunis and Samaha in 1971 (14), in muscle biopsy specimens from two patients with chronic polymyositis led the way for further recognition of "inclusion body myositis." This term was coined by Yunis and Samaha, who described a 26-year-old woman who developed slowly progressive wasting and weakness of the proximal muscles; inflammatory infiltrates were exhibited in her muscle biopsy and nucleocytoplasmic filamentous inclusions in her electron microscopy. Detailed clinical and microscopic descriptions of this disease were given by Carpenter et al in 1978 (15) and by Danon et al in 1982 (16).

The clinical and laboratory findings in our patients are similar to those reported previously (Table 2). One unusual clinical finding is profound atrophy, out of proportion to that of other affected muscles, of certain muscle groups (quadriceps femoris) in our patients, in the patient of Vital et al (21), and in three patients of Danon et

TABLE 2

Comparison of Characteristics of Inclusion Body Myositis and Other Forms of Inflammatory Myopathy

	Myositis	Other forms of inflammatory myopathy
Age of onset	Usually sixth decade	Variable
Sex	No definite predilection	No definite predilection
Family history	Negative	Negative
Clinical course	Very slow, protracted	Subacute or rapid
Distribution of weakness	Either mainly proximal or distal sometimes generalized	Mainly proximal
Extraocular muscles	Spared	Spared except when cranial nerves are involved, as in sarcoidosis
Dysphagia	Variable	Frequent, especially in polymyositis or dermatomyositis
Myalgia	Usually absent	Usually present
Muscle mass	Affected muscles atrophic	Diffuse atrophy late in course or when complicated by steroid atrophy; may be hypertrophic in sarcoid myopathy
Creatine kinase	Usually mildly elevated	Moderately to markedly elevated
Collagen vascular disease and immunologic abnormalities	Absent except in two cases (17, 18)	Frequent
Malignant neoplasm	Absent except in pt 6 of Carpenter (15)	Present in 15%–34% (4)
Virologic studies	Negative (19) except in 1 case (20)	Variable results (2, 4) (some cases with "viral particles" were actually cases of inclusion body myositis
Response to immunosuppressive therapy	Poor	Generally good
Muscle biopsy		
Light microscopy	Mononuclear cellular infiltrates of varying density, numerous vacuoles lined with basophilic granules, occasional enlarged nuclei with peripherally displaced chromatin and eosinophilic inclusion	Characteristic vacuoles not present; giant cells and granuloma may be seen (9–11)
Ultrastructure	Numerous cytomembranous bodies and aggregates of nucleocytoplasmic filaments	Nonspecific degenerative changes
Electrodiagnosis	Positive sharp waves, fibrillation potentials, and mixed "myopathic" and neuropathic motor units; normal nerve conduction velocities	Similar findings except when complicated by neuropathy
Postmortem: spinal cord and nerve roots	No data available to date	Normal except in collagen vascular disease or sarcoidosis

al (16). Although this could be attributed to steroid treatment, our second patient had not received steroid therapy previously. It is possible, however, that the diabetes mellitus in this patient might have contributed to his quadriceps atrophy.

Electrodiagnostic testing in inclusion body myositis has yielded mixed findings consisting of small- and short-duration, easily recruitable motor units ("myopathic") and long-duration, high-amplitude motor units (neuropathic) (15, 16, 22). Frequently, increased insertional activity, positive sharp waves, and fibrillation potentials, all indicative of active muscle disease, have been demonstrated. Nerve conduction velocities are usually normal.

Histologically, the clue to diagnosis is the presence of vacuoles lined with bluish granules within muscle fibers in hematoxylin-and-eosin-stained cryostat sections. Since the basophilic granules are removed by lipid solvents, paraffin sections will not demonstrate them and leave only the vacuoles and cellular infiltrates. It is possible that previous patients diagnosed as having idiopathic polymyositis but who proved resistant to steroid therapy and were studied only by paraffin sections were cases of misdiagnosed inclusion body myositis.

Ultrastructurally, this form differs from other types of myositis by virtue of massive collections of cytomembranous whorls (pseudomyelin structures), nucleocytoplasmic filaments within the sarcoplasm of affected muscle fibers, and preservation of the capillary network. The significance and origin of these filaments and their relationship to muscle destruction are unknown. They were formerly believed to be viral products released from disintegrating nuclei, but virologic studies have been unrevealing (19) except for isolation of an adenovirus type 2 from a muscle specimen of a 39-year-old woman (20). Similar filaments have been reported in nuclei of osteoclasts of patients with Paget's disease (23) and giant-cell tumors of bone (24). In Patient 2 of Danon et al (16), muscle weakness developed at the time he was found to have clinical and radiologic signs of Paget's disease. The concurrence of those two disorders is intriguing.

None of the histologic and ultrastructural findings we report here are unique to inclusion-body myositis (15, 21). Similar vacuoles have been described in oculopharyngeal dystrophy (1, 25); however, in inclusion-body myositis the rimmed vacuoles are far more bountiful, and the abnormal filaments are much larger (25). Membranous whorls have been observed in various myopathies, such as adult-onset acid maltase deficiency and toxic myopathies due to chloroquine, colchicine, and vincristine, but are not encountered in other types of inflammatory myopathy (15).

The inclusion-body form is probably more common than the literature suggests, but its etiopathogenesis, whether a primary myopathic or a neuropathic disease (26), remains to be elucidated. Postmortem examination of the spinal cord and nerve roots might be useful. Despite group atrophy, we agree with Carpenter et al (15) and Danon et al (16) that inclusion body myositis is a primary disease of muscle because of structural changes in muscle (vacuolation, cellular infiltrations, degenerative and regenerative changes, and tubulofilamentous inclusions) not ordinarily seen in denervating disorders. We suggest that when the diagnosis is polymyositis, before therapeutic trial is undertaken, a complete histochemical and ultrastructural study of skeletal muscle biopsy and, if applicable, a quantitative assay for muscle enzymes be performed to rule out other neuromuscular disorders. Such diagnostic laboratory investigations must be completed satisfactorily before meaningful interpretation of treatment response or prognostication can be made.

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Environmental Medicine Notes

Alf Fischbein, editor

Despite recent standards promulgated by the Occupational Safety and Health Administration of the U.S. Department of Labor to prevent occupational lead poisoning, it is still a common occupational disease in the United States. Concern also centers on the association between exposure to lead in the ambient environment of industrial nations and disturbed mental development in children. The study detailed below calls attention to lead exposure at its worst: occupational exposure of children and women. The paper demonstrates the potential hazard to future generations of currently uncontrolled work conditions, and points to the urgent need for assigning high priority to insuring occupational safety and health in developing countries—AF.

Blood Lead and Erythrocyte Protoporphyrin Levels Among Papiér-Mâché Workers in Kashmir

PRABHA SHARMA KAUL, M.D., AND BALKRISHENA KAUL, PH.D.

Abstract

In order to examine the extent of undue lead absorption in papier-mâché workers, blood lead and erythrocyte protoporphyrin levels were measured in a group of 30 papier-mâché workers in Kashmir, India. The mean blood lead level of exposed workers was 69 μg/dl, compared with 16 μg/dl for a control group. Erythrocyte protoporphyrin levels were 116 μg/dl and 30 μg/dl for the two groups, respectively. These results suggest that monitoring of erythrocyte protoporphyrin and/or blood lead levels of workers employed in this trade is urgent, particularly since the majority of the workers are women of childbearing age, and because children are part of the training and the work force.

The art of papier-mâché, practiced in the East for many centuries, came into prominence in Europe in the middle of the eighteenth century, principally in the form of decorated trays, boxes, toys, and other domestic articles. These articles are composition derived from waste scrap paper pulp, resin, glue, fine chalk, clay, drying oils, and sand molded into various objects (1). The dried smooth surface of the articles is painted with assorted designs using single or combined dyes. White lead (lead acetate) constitutes the principal ingredient of all color mixtures used in this trade.

Lead poisoning is a prevalent disease in many industrialized countries (2, 3). Occupations associated with exposure to lead include primary lead smelting, recovery of scrap, cutting of steel coated with lead-containing paint, manufacture

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of automobile batteries, cable splicing, paint and pigment handling, and stained glass manufacturing. The major route of lead absorption in occupational settings is by inhalation of lead dust and fumes, but ingestion from contaminated food and cigarettes can also contribute to excessive lead exposure (4).

Long-term occupational exposure to lead has been shown to be associated with anemia and neuropathy; severity of symptoms has been correlated with both blood lead levels and zinc protoporphyrin levels (5, 6). Occupational lead exposure has also been associated with central nervous system dysfunction and neurologic impairment, ranging from subtle derangement of behavior, intellect, and peripheral motor function, to gross encephalopathy (7, 8). Lead may also be toxic to the reproductive system (9).

Lead-related biochemical effects on heme biosynthesis have recently been translated into useful laboratory diagnostic tests (10).

In order to assess degree of lead absorption and effect on erythrocyte protoporphyrin levels in a group of Indian workers, a survey of 30 papier-mâché workers and trainees in Kashmir, India was conducted in August 1981. Excessive exposure was suspected because of poor industrial hygiene, such as licking the brush tip during painting and inhalation and possible ingestion of dust when preparing the powdered paint.

Materials and Methods

A group of 30 (21 females and 9 males) occupationally exposed trainees and workers ranging in age from 10 to 70 years were screened and compared with 20 control subjects aged 20–60 years, not known to be occupationally exposed to lead. The mean age of female workers/trainees was 19 years, the median was 22 years, males had a mean age of 32 years with a median of 23 years. For the control group, the mean was 37 years and the median was 32 years. Finger-prick blood specimens were collected by scrubbing the subject's finger for approximately 10 sec with a prepackaged alcohol swab. After drying, a film of flexible colloidion was applied and the finger punctured after the colloidion had dried; at least five spots (approximately ⅜ in. diameter) of blood were collected on filter paper disks (No. 903, Schleicher and Schuell, Keene, NH). These were packed and sealed immediately between layers of weighing paper sheets (Fisher Scientific, Pittsburgh, PA) until the time of analysis. One disk each was punched out from the blood-soaked area for free erythrocyte protoporphyrin (EP) by extraction method, two disks for duplicate blood lead (Pb-B) determinations using the Delves-Cup procedure by atomic absorption spectrophotometry, and one disk for hemoglobin determination by extraction method. Dye powders were analyzed by atomic absorption spectrophotometry using the Hessel extraction procedure (11–14).

Results

The mean Pb-B level of the occupationally exposed group was 69 μg/dl, and the mean Pb-B level of the control group was 16 μg/dl (Table). Differences were also observed between EP levels in the two groups. The mean EP concentration of the study group was 116 μg/dl, significantly higher than the mean of 30 μg/dl observed in the nonexposed control group. The difference in the mean hemoglobin level of the exposed and control group was not significant (14.6 gm% in control subjects versus 11.8 gm% in the exposed group).

The analytical data of the various powder dyes used in this handicraft showed a potential for excessive inhalation; the amount of lead in the products ranged between 0.025 gm% (Ultramarine, Artineel) to 83 gm% (Red Lead, Cindoor).

Discussion

The craft of papier-mâché is ancient and a source of income to a significant segment of the handicraft industry of the Jammu and Kashmir State. As far as the authors are aware, the articles made are used only for decorative purposes, but may occasionally be used to store such items as dried fruits, cigars, soap, cosmetics, and jewelry. No study has been conducted with regard to lead contamination of items stored in such articles, but it is conceivable that these could be a potential source of lead exposure if items are ingested, particularly by children.

The first clinical case report of lead poisoning among papier-mâché workers from Kashmir was recently documented (15). Our report represents the first confirmation of lead poisoning in a larger group of such workers and trainees. Since this screening was conducted in a field setting, a certain degree (10%–20%) of lead contamination inherent to this type of sample collection and technique cannot be excluded and may be supported by the relatively low correlation between EP and Pb-B, notably $r = 0.27$ (16). However, in the absence of detailed occupational histories with regard to duration of exposure, this correlation may also reflect variable exposure to lead and iron-deficiency incidence (10).

TABLE

Blood Lead (Pb-B), Erythrocyte Protoporphyrin (EP), and Hemoglobin (Hgb) Levels in Papier-Mâché Workers and Controls

	Papier-mâché workers (*n* = 30)			Controls (*n* = 20)		
	Pb-B (μg/dl)	EP (μg/dl)	Hgb (gm %)	Pb-B (μg/dl)	EP (μg/dl)	Hgb (gm %)
Mean	69.1 (23–122)*	116.5 (5–528)*	11.8 (8.4–16.3)*	16.2 (9–36)*	30.5 (5–168)*	4.6 (0.5–14.2)*
Standard deviation	25.5	132.6	1.6	7.1	45.3	2.7
Geometric mean (GM)	64.4	67.1	11.7	15.0	15.7	13.7

* Range.

The mean EP level of 116 μg/dl among exposed workers is significantly higher than 30 μg/dl for the control group. According to the U.S. Center for Disease Control, EP values in excess of 35 μg/dl are indicative of lead poisoning and/or iron deficiency in a pediatric population (17). Since the mean Pb-B of the exposed workers is significantly higher than that of the control group, much of the rise in EP values is probably a direct biological response to elevated levels of Pb-B, although measurement of serum iron was not performed.

The elevated Pb-B levels in female workers of childbearing age is a matter of great concern. Previous studies have shown that newborns exhibit similar or only slightly lower Pb-B than their mothers (18). A recent study has associated prenatal exposure to low levels of lead with retardation of an infant's mental development (19). As umbilical cord blood lead levels increased, intellectual performance at 12 months of age decreased. Levels as low as 12 μg/dl in the newborn were hazardous, as evaluated by the standard Bayley scale of infant development. This presents a serious public health problem in that occupationally exposed young females, such as those surveyed in this study, could have toxic levels of lead adversely affecting the development of a variety of organs in their fetuses. It would be important to measure and compare Pb-B levels of occupationally exposed expectant mothers and their newborns to determine effects on neurologic and mental development, as was recently done in an environmentally exposed population in Europe (20).

During our visit to various work sites for blood collection, workers were made aware of the hazards of lead exposure and the importance of observing careful personal hygiene practices in their trade. The exposure of minors, who constitute the majority of the examined trainees, should particularly be recognized. Urgent preventive measures and medical surveillance are warranted. In a setting like the one described here, this could perhaps be done by measurement of EP, followed by measurement of Pb-B, if the EP was positive. Although various standards for maximum blood lead levels have been proposed, a Pb-B of 30 μg/dl and higher and an EP level of 50 μg/dl or more by Piomelli's procedure or 35 μg/dl or more by hematofluorometers should be considered indicative of undue lead absorption and/or iron deficiency (21).

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Pharmacology Notes

John P. Morgan, editor

This is the first in an occasional series of notes on pharmacology for The Mount Sinai Journal of Medicine. The notes will not focus on hospital practice but on therapy and drug use in general. Although I promise to attempt the practical in these notes, they will reflect my interest in the social and political context of drug therapy as well. Future notes in 1986 will include a review of AIDS in drug abusers and a speculative note on therapeutics in Alzheimer's disease.—JPM.

Characterization and Treatment of Dependence on Tobacco Cigarettes

John P. Morgan, M.D.

The habitual use of tobacco cigarettes surely constitutes drug dependency, but its unusual character merits careful commentary. The varying rituals of tobacco consumption have heretofore clouded the most likely truth, that they constitute a variety of approaches to deliver nicotine to the brain. The minimal disruption of normative social behavior by nicotine contributed to a widespread social acceptance and encouragement of use. Tobacco is the only dangerous drug vehicle without medicinal utility, whose agricultural production is subsidized by both federal and state governments. Nicotine is a powerful pharmacological agent which in some circumstances of ingestion may cause death. Although chronic cigarette smokers apparently suffer minimal tissue toxicity from the nicotine itself, they can suffer grievous damage from the coingestants generated by the combustion of tobacco and the inhalation of the smoke. One might properly draw a parallel to the intravenous heroin user who under current circumstances of use suffers little chronic toxicity from heroin itself but because of bacterial, viral and pharmacologic contaminants is damaged by the processes of ingestion.

Although the realization of the danger of tobacco-related pulmonary and cardiovascular disease is one force in the growing opposition to cigarette smoking, a moral distaste and anger at exposure to smoke is also operative. Generally, when moral distaste at any drug use enlarges, the culture seeks material and biomedical justification. Hence the taxi driver displays a sign that he is "allergic" to cigarette smoke. We as a society have also emphasized the very uncertain possibility that passive inhalers, such as spouses and theater-lobby occupants, are harmed by their exposure to low doses.

The warning on the cigarette package has evolved from reference to the authority of the Surgeon General to warnings about carbon monoxide. What this might mean to the smoker is unassessed. There have been recent appeals to change the warning further to add that cigarette smoking is "addictive." Whether this nasty warning would dissuade anyone is also unclear.

Finally, there is a continuum of dosage among smokers ranging from a few cigarettes to a few packs per day. However, nondependent consumption—occasional, infrequent use—is apparently a rare pattern. Ingestion of nicotine is very seductive and users apparently have great difficulty not becoming dependent and engaging in repeated high dose use (1).

The Pharmacology of Nicotine

Nicotine was isolated from tobacco in 1828. The average cigarette contains 15–25 mg of nico-

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tine; however, loss in smoke is high, and consumption per cigarette averages less than 3 mg. The smoke of filtered cigarettes may deliver between 0.2 and 1.0 mg. Although tobacco is the most common source, some powdered home garden insecticides are nicotine based. Despite the drug's potential lethality, reported deaths are relatively few. Ingestion of insecticide or the eating of cigarettes by children may be lethal. (The estimated fatal dose is only 30–60 mg.) However, nicotine is poorly bioavailable from the gastrointestinal tract and the strong emetic action is protective as well. I shall not review here the constellation of symptoms that may be caused by nicotine's ability to stimulate autonomic ganglia and neuromuscular junctional sites (2).

Nicotine in inhaled cigarette smoke is very well absorbed from the alveolar membrane. Nicotine in cigar and pipe smoke, which is more alkaline, is well absorbed from the buccal mucosa in this form but still results in lower serum concentrations than those found in cigarette smokers. The process of flu-drying, in which tobacco is exposed to forced hot air, produces both an acidic form of nicotine and a smoke which is less harsh and easier to inhale. Nicotine is also readily absorbed via chewing tobacco which is held against the buccal mucosa for a prolonged time.

The ignition of tobacco produces literally thousands of compounds, including carbon monoxide, ammonia, nitrosamines, ketones and "tars," polycyclic aromatic hydrocarbons which are carcinogenic. The adverse health consequences are more likely related to these co-ingestants than to nicotine.

Reinforcing Character

Nicotine in humans has alerting and muscle-relaxing effects. Its character as a stimulant is quite perceptible to smokers and when given intravenously is accorded by smokers as pleasurable and shows increased scores similar to morphine and amphetamine on a series of rating scales derived by The Addiction Research Center (3). It seems likely that most cigarette smokers are titrating their dosage of nicotine to provide an adequate serum level to deliver the drug to the brain. If low-dose nicotine cigarettes are substituted, smokers puff more often, draw more deeply, and smoke the cigarette to a small butt. Conversely, they become less efficient smokers when high-dose material is offered. In double-blind studies, smokers given nicotine intravenously decrease their ingestion of cigarettes compared to those given an intravenous placebo. This pattern of titration is of obvious importance in the era of low-nicotine, low-tar cigarettes. The appetite for nicotine may lead the smoker to inhale more damaging coingestant to maintain a desired serum concentration of nicotine.

Drug Dependence

Tolerance to some nicotinic effects occurs; however, the regular user still responds to smoking with increased blood pressure and pulse rate, muscle tremor, and decreased skin temperature. Even the limited tolerance is not persistent, and the first cigarette of the morning produces greater subjective and pharmacologic effect than later doses.

Most clinicians (and certainly most smokers) agree that a strong craving for tobacco emerges on cessation of smoking. This withdrawal regularly includes anxiety, restlessness, and impaired concentration. Some complain of drowsiness, headaches, and insomnia and, often, increased appetite; a return to normal appetite may also occur. Physiological concomitants accompanying withdrawal include EEG changes, decreased performance in some psychomotor tests, and decreased pulse rate and blood pressure (4).

Treatment

The model presented here would indicate that in an approach to nicotine use, dependence, and abstinence, drug replacement might have a place. Most smokers wish to stop and many have switched to low-tar, low-nicotine brands. Many forms of smoking-cessation programs, including individual and group counseling and a variety of such uncertain interventions as hypnosis, acupuncture, and conditioned avoidance, have been tried. Most smokers who try to stop have good short-term success but—like other drug dependencies—remissions and relapse are likely. Most surveys have shown that structured programs have only a 1-in-5 success rate after one year. Despite these pessimistic outcomes, millions of Americans, including this author, have managed to stop after a number of unsuccessful tries. Importantly, most stopped with no particular help, pharmacologic or social.

Nicotine Replacement

Nicotine will relieve the withdrawal symptoms of a stopping smoker. A related alkaloid, lobeline, has been available for years as a replacement but probably is not effective. Orally administered nicotine is subject to significant hepatic metabolism and has no clinical utility.

A sugar-free chewing gum containing a cation-exchange resin loaded with 2 mg of nicotine became available on prescription under the name Nicorette (Merrell-Dow) in the United States in late 1984. A 4-mg dosage form available in other countries has not been released here. The product incorporates bicarbonate buffering that insures protonation of the nicotine to an alkaline form, enhancing buccal mucosal absorption. Ninety per cent of the available nicotine is absorbed at a relatively slow and uniform rate. Chewing the gum does not mimic the rapid serum rise in nicotine produced by inhaled cigarette smoke, and chewing one piece of gum per hour produces serum concentrations approximately half of those following smoking one cigarette per hour. Early trials documented that withdrawal symptoms were lessened, and at least 15 controlled double-blind studies have indicated that the product had effect in maintaining a cigarette-free state. In terms of absolute cessation, the gum increases the one-year-free rate by 5%–20% (5–8). In one study published in JAMA, after one year of placebo gum and psychosocial counseling, 15% of previous smokers remained abstinent, whereas approximately 30% of the nicotine-gum users remained abstinent (9).

Patients are best instructed to chew the gum slowly when they feel the urge to smoke. Most will use 10 to 15 pieces per day, but some will approach the recommended 30 pieces per day. The most common side effects are hiccups, nausea, vomiting, and headache. These symptoms may diminish with time and may be diminished by slowing chewing and decreasing the number of pieces. Anecdotal reports of loosened dental fillings are common. Approximately 5% of patients will continue to use the product for a prolonged period of time, as if they had switched dependence to the gum. Nicotine gum is contraindicated in individuals with recent myocardial infarction, arrythmia, or vasospastic disorders.

Recent experimental data indicate that nicotine prepared in a nasal spray yields higher serum concentrations and may be another effective method of drug delivery therapy (10).

Nicorette (Merrell-Dow) is available in boxes of 96 pieces at an approximate cost to the consumer of $30.00. An individual using 10–15 pieces a day will pay approximately $100.00 a month. For comparison, a 2–2½ pack-per-day cigarette smoker will spend approximately the same amount a month on cigarettes, at least in New York City.

If the American experience approximates that of other countries, 80% of practitioners will be asked to prescribe Nicorette in any three-month period by smokers who wish to quit. Although the product may be more useful in the supportive setting of smoker-cessation clinics than in the practitioner's office, I believe that many patients so requesting should be given a therapeutic trial of this unusual drug product.

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Radiology Notes *Jack G. Rabinowitz, editor*

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A Case of Meconium Peritonitis with Ileal Stenosis: Prenatal Sonographic Findings with Radiologic Correlation

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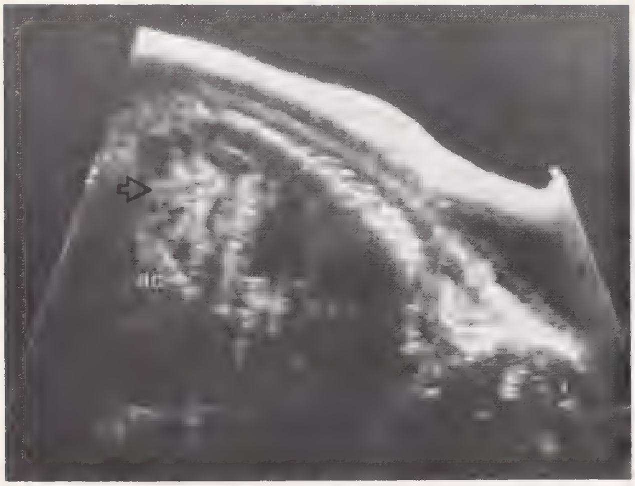
Case Report. A 20-year-old Hispanic woman, gravida 1, para 0, demonstrated a single fetus in a vertex presentation in a routine last-trimester sonogram. The fetal abdomen, however, showed several dilated fluid-filled bowel loops with low-level echoes presumed to be meconium. A solid mass with strong dense echoes accompanied by calcium acoustic shadowing was also identified in the lower abdomen (Figs. 1, 2). The presence of distended bowel loops with a calcified mass was diagnostic for meconium peritonitis associated with distal small-bowel obstruction.

Following spontaneous labor, a normal male infant was delivered. The infant soon developed a distended abdomen, and at the age of 16 hr still had not passed meconium. A radiograph of the abdomen confirmed the prenatal sonographic findings and showed no evidence of free air (Fig. 3). A barium enema was initiated, and a microcolon with a normally positioned cecum was observed (Fig. 4). A laparotomy was performed, and in the right iliac fossa, a mass containing multiple adhesions, bands, and calcifications was found. The distal ileum was narrowed and the proximal right colon and 6 in. of the distal ileum were resected. An ileostomy and a right mucous colonic fistula were constructed. The sweat chloride test was normal.

Discussion. Meconium peritonitis is a sterile chemical peritonitis that occurs following spillage of meconium into the peritoneum. The majority of cases of meconium peritonitis are asso-



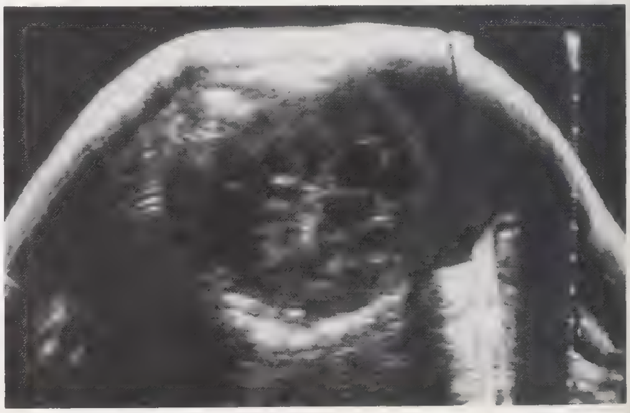


Fig. 1. **Left** Longitudinal sonogram of fetus demonstrates solid mass in lower abdomen with dense echoes (arrow), accompanied by posterior acoustic shadow (ac).

Fig. 2. **Right** Transverse sonogram of fetus at level of midabdomen. Several dilated, fluid-filled bowel loops are seen.

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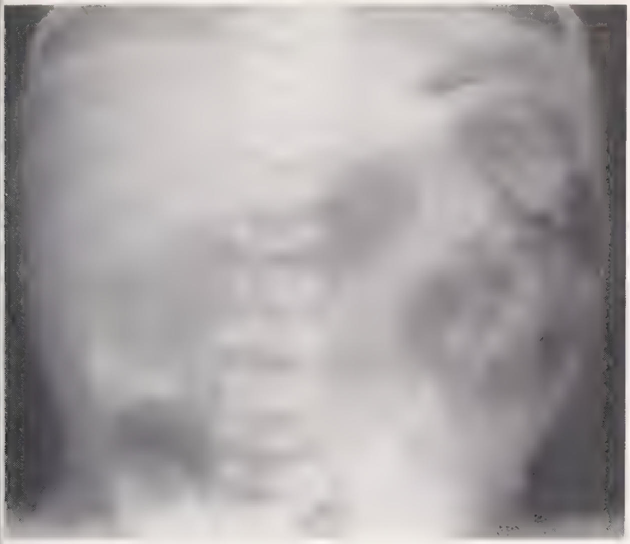

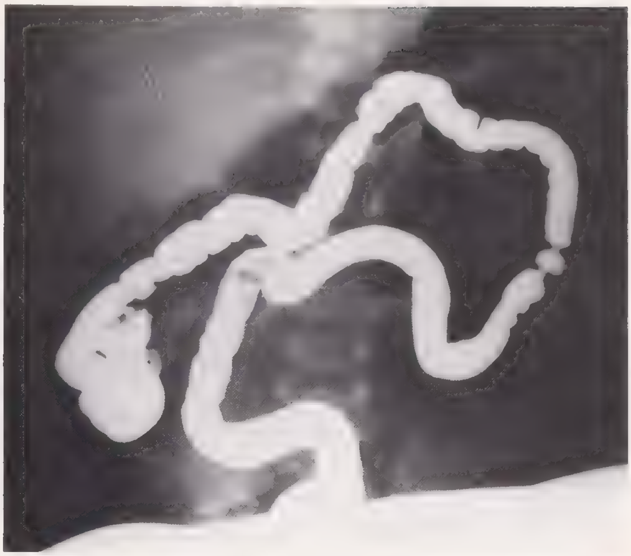

Fig. 3. Left Abdominal radiograph shows calcification within right lower quadrant of abdomen and distended loops of bowel.
Fig. 4. Right Barium enema demonstrates microcolon.

ciated with perforation although, not infrequently, no obvious site can be found. Meconium calcifies quickly when in contact with peritoneal fluid and produces extensive fibroadhesive changes with calcifications. Fifty percent of cases of meconium peritonitis have been reported to be associated with obstructive lesions of the small bowel (1, 2).

An intrauterine vascular catastrophe in the well-developed small bowel is now recognized to be the main cause of atresia or stricture (3). The bowel distends proximal to the occlusion, and in some patients perforation may occur. The perforation often seals off before birth, which most likely happened in the case presented here. Severe inflammatory changes occurred in the vicinity, resulting in a mass containing adhesions and calcifications. However, if the communication with the intestine remains patent, cystic meconium peritonitis results (4).

Real-time sonography is becoming a valuable modality in diagnosing abdominal masses in utero. The mass detected in the case presented here was solid and nonhomogeneous, since it contained meconium adhesions and calcific plaques. Distended, fluid-filled loops of bowel were identified. These findings are diagnostic of meconium peritonitis with small-bowel obstruction. A barium colon examination performed after birth demonstrated a microcolon, indicating the presence of a distal ileal obstruction, since little succus entericus passes the area of obstruction.

Differential diagnosis of a calcified mass in a fetal abdomen should include retroperitoneal teratoma or calcified neuroblastoma. Consequently, the association of the mass with several dilated loops of bowel and microcolon is important, suggesting the diagnosis of meconium peritonitis with distal bowel obstruction. The prenatal sonographic diagnosis is valuable in the proper surgical and pediatric management of the infant.

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Posterior Mediastinal Masses in Two Siblings with Hemoglobin E-β Thalassemia

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Case Report. The two patients presented here, a 21-year-old man and a 17-year-old girl, are siblings of Oriental origin with hemoglobin E-β thalassemia who have been receiving four to five units of blood per year since the age of nine. The father is known to have severe anemia, and the mother to be phenotypically normal. Of the five offspring, the remaining three are not affected by the disease.

Physical examination revealed short, pale, jaundiced patients with absence of secondary sex characteristics and frontal bossing. Hepatosplenomegaly was present and gallstones were noted in the sister. Hemoglobin levels ranged between 5 and 7 gm/dl. Poikilocytosis, anisocytosis, and microcytic hypochromic anemia were noted. Hemoglobin electrophoresis showed a high amount of hemoglobin E and F, without hemoglobin A, characteristic of hemoglobin E-β thalassemia.

X-ray studies demonstrated widening of the diploic space with spicules of bone oriented in the characteristic radial pattern known as a "hair-on-end" appearance. These changes were absent inferior to the internal occipital protuberance. Except for the ethmoid sinus, all the sinuses were obliterated (Fig. 1).

Chest radiographs of the sister, similar to those of the brother, revealed marked osteoporosis and widening of the shaft of the ribs with lobulated masses extending from the second to the tenth thoracic vertebra within the posterior mediastinum (Fig. 2). Hand radiographs obtained on the brother showed cortical thinning and widening of the bones with a coarse trabecular pattern. All the epiphyses at the wrist were still unfused, indicating severely delayed bone maturation.

The siblings are now being treated with Dexferroxamine in addition to vitamin K and folic acid.

Discussion. Hemoglobin E-β thalassemia is a rare hemolytic anemia known to occur in southeast Asia. Hemoglobin E is found primarily in the Oriental population. Interaction between the two genes of hemoglobin E and β thalassemia produces severe anemia similar to thalassemia major (1). Both our patients recently emigrated to this country from Hong Kong. Thalassemia major and hemoglobin E-β thalassemia are similar radiographically, since both cause excessive marrow proliferation. Erythroid hyperplasia of bone marrow is striking in the skull. As a result, the diploic space becomes widened and the outer table thin, owing to pressure atrophy. When the trabeculae orient themselves perpendicular to the tables, the classical "hair-on-end" pattern results. The sinuses are obliterated as a result of marrow overgrowth of the facial bones.

Heterotopic marrow can frequently be found as microscopic foci in the liver, spleen, and lymph nodes. Extramedullary erythropoiesis can also present as tumorlike masses in the posterior mediastinum, peritoneum, or pelvis. Central nervous system involvement has also been described in patients with hemoglobin E-β thalassemia (2, 3). The paravertebral soft-tissue masses noted in the patients described here indicate long-standing hemolytic anemia for which supportive treatment was given for many years (4).

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The origin of these masses is thought to be due to extrusion of marrow through a defect at the thin anterior cortex of the widening ribs. This defect is most severe in the posterior part of the rib and explains the more frequent posterior mediastinal location of extramedullary erythropoiesis (5). Similar occurrences, however, have been found in patients with thalassemia, adjacent to the anterior end of the ribs where the bony changes were most prominent (6). Microscopically, these masses are enclosed within a capsule formed by periosteum (5) and consist of active hematopoietic tissue continuous with the marrow of the adjacent rib.

Extramedullary erythropoiesis is associated with most severe hereditary hemolytic anemias, with myelofibrosis, and with extensive infiltration of bone by neoplastic processes. The differential diagnosis of posterior mediastinal masses includes neurogenic tumors, thoracic spine abscesses, intrathoracic meningomyelocele, and other primary or secondary malignancies. The presence of soft-tissue paravertebral masses in association with other characteristic bony changes should strongly suggest the diagnosis of hemoglobin E-β thalassemia.

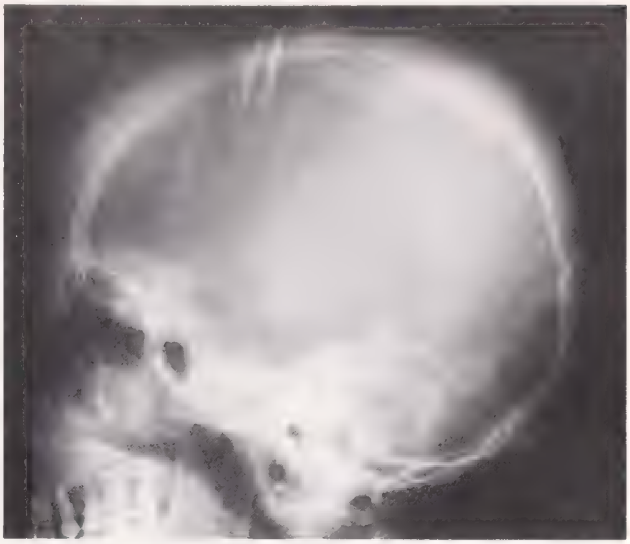

Fig. 1. Lateral projection of skull shows widening of diploic space with characteristic "hair-on-end" appearance. All sinuses are obliterated except for ethmoid sinuses.

It is of clinical importance to recognize this association, both for differential diagnositic purposes and to avoid the possibility of sudden neurologic problems. Proper diagnosis and prompt and sufficient treatment of the underlying

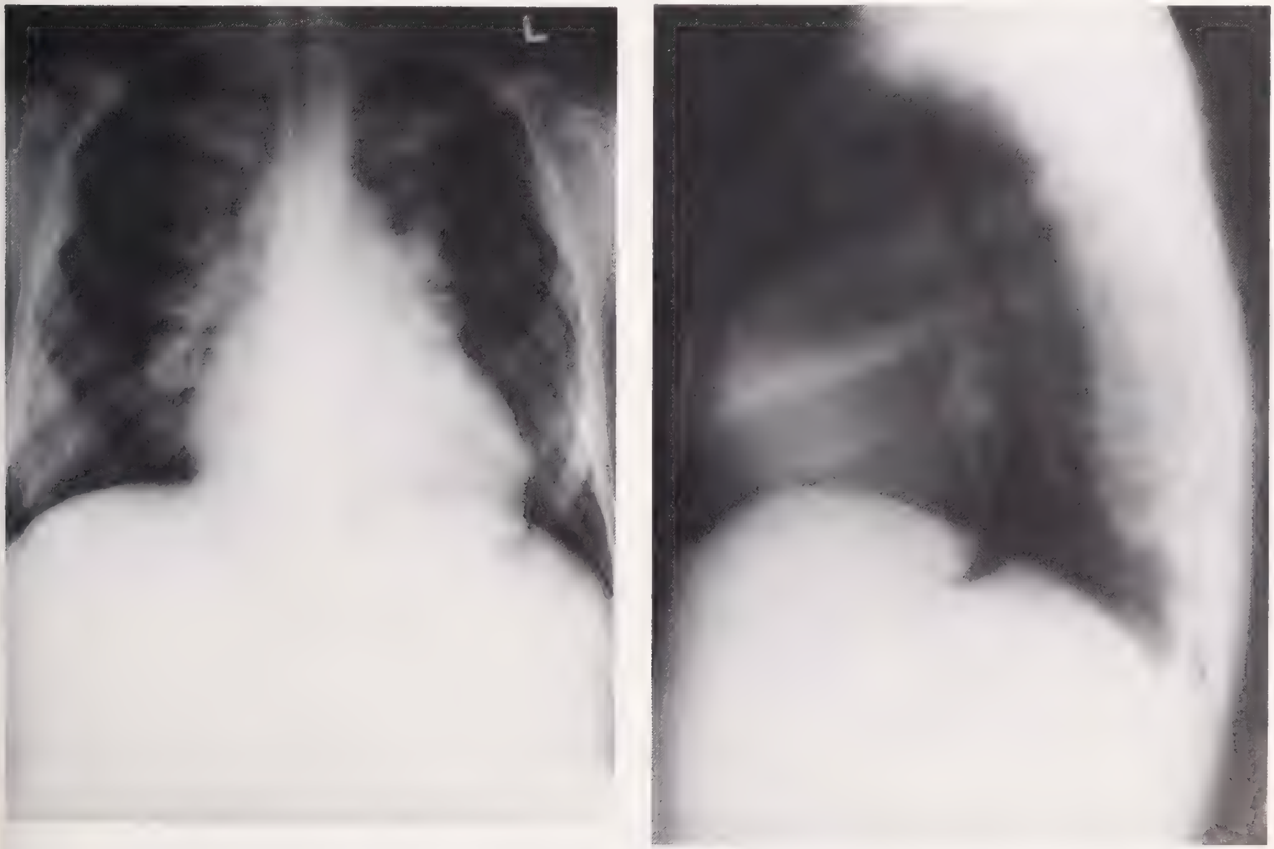

Fig. 2. Chest radiographs demonstrate broadened ribs with cortical thinning. Lobulated posterior mediastinal masses of extramedullary erythropoiesis are seen extending from T-2 to T-10.

anemia will help retard and prevent extensive growth of extramedullary hematopoietic tissue.

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Radiology Notes *Jack G. Rabinowitz, editor*

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Mediastinal Hematoma in an Intravenous Drug Abuser

M. V. RAMANI RAO, M.D., THOMAS C. SNYDER, M.D., BENITO RYBAK, M.D., AND ULRICK VIEUX, M.D.

Case Report. A 22-year-old woman with a long history of intravenous drug abuse came to the emergency room because of chills, drenching sweats, and a firm, tender erythematous mass in her right groin. The patient had recently been treated with oral antibiotics for a neck abscess.

On examination, she was febrile to 102°F. Scars on the neck and the abscess in her right groin were apparent. A grade 2/6 systolic murmur was noted. There was no evidence of peripheral emboli. The laboratory values consisted of hemoglobin 10 gm/dl, hematocrit 30.9%. The white blood cell count was 15,700 with a shift to the left: polymorphs 64%, bands 3%, lymphocytes 25%; monocytes, 3%; eosinophil, 5%. Chest X-ray demonstrated a left-sided mediastinal mass adjacent to the aortic arch (Fig. 1). A presumptive diagnosis of subacute bacterial endocarditis and possible mycotic aneurysm of the aorta was considered, and the patient was admitted to the hospital for further evaluation.

During the hospital stay, all blood cultures were negative, and an echocardiogram was normal. Computerized tomography of the mediastinum (without intravenous contrast owing to poor veins and patient's refusal) revealed a left anterior mediastinal mass adjacent to the trans-

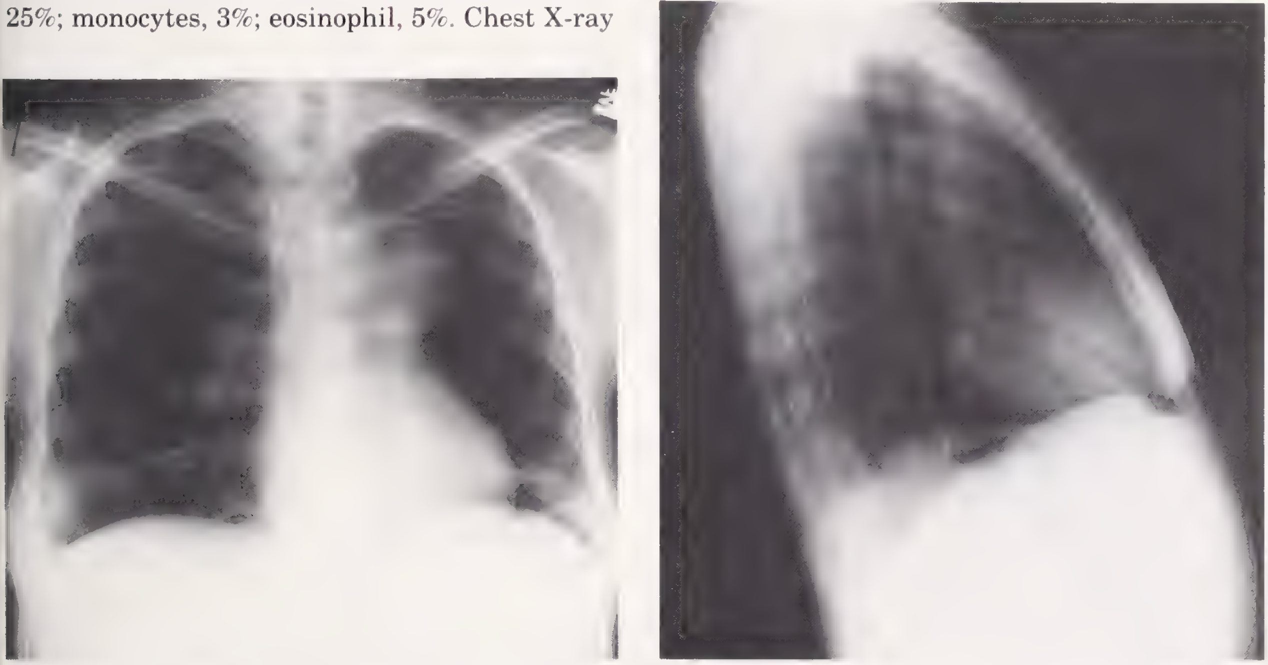

Fig. 1. Frontal (**left**) and lateral (**right**) projections of chest show mass in left anterior (A) and middle mediastinum (B). Aortic knob is well seen to right of mass.

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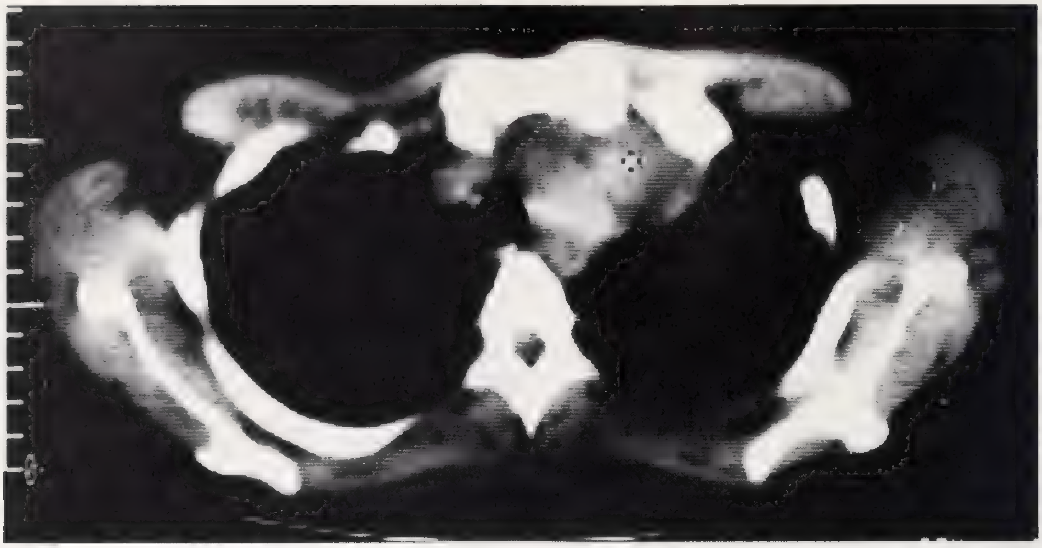

Fig. 2. Computerized tomography of chest without contrast demonstrates mass (asterisk) adjacent to but not involving aorta.

verse aortic arch (Fig. 2). An arch aortogram, performed to exclude an aortic aneurysm, was normal (Fig. 3) except for the incidental finding of an aberrant right subclavian artery. A mediastinotomy was performed following an unsuccessful mediastinoscopy.

A hemispheric mediastinal mass measuring approximately 3.5 × 2.0 cm was discovered. On one surface the mass was convex and on the opposite surface, concave. Upon sectioning, the mass contained dark red and yellow laminated friable tissue surrounded by a thin capsule. Histologically, the lesion was comprised of vascularized fibrous tissue infiltrated by lymphocytes and hemosiderin-laden macrophages that surrounded an unorganized hematoma.

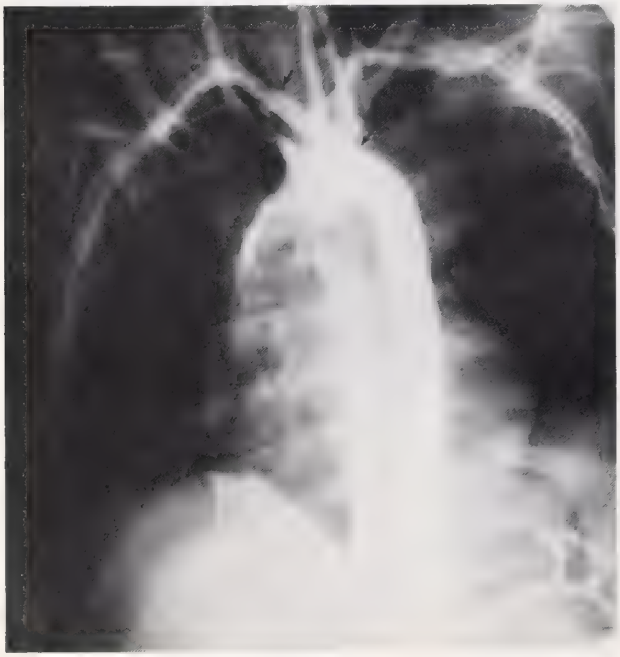

Fig. 3. Arch aortography in RPO projection reveals normal aorta and mass adjacent to arch. Aberrant right subclavian artery (arrow) is also present.

Discussion. Mediastinal hematoma is an unusual complication of intravenous drug abuse. Vascular complications already known to be associated with drug abuse include thrombophlebitis (1), bacterial endocarditis, emboli, arteriovenous fistula, mycotic aneurysm (2), and hemothorax (3). Although this mass was originally thought to represent an aneurysm, closer observation on the chest film shows a normal-appearing aortic knob.

Mediastinal hematoma more commonly occurs following severe blunt or penetrating chest trauma. It has also been associated with thoracic surgery, dissecting aortic aneurysm, coagulation compromise, uremia, infection, or bleeding into a mediastinal cyst or tumor. An unusual pathway is extension of blood from the soft tissues of the neck into the mediastinum following trauma or associated with coagulation disorders (4).

The pathogenesis of the mediastinal hematoma in the patient presented is thought to have pursued this route following laceration of a blood vessel during an attempted intravenous injection into the neck. Therefore, hematoma should be included in the differential diagnosis of a mediastinal mass. In general, the differential diagnosis of any mass in the anterior and middle mediastinum consists of lymphoma, thymoma germinal cell neoplasm, primary and metastatic carcinoma, neural tumors, mediastinal abscess, granuloma, hematoma, and bronchogenic and enteric cysts (5). However, the history of intravenous drug abuse with obvious past use of neck veins should suggest this diagnosis.

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"It would seem that we are warranted in stating that the invasion and dissemination of benign endometrial tissue employ the same channels as the invasion and dissemination of cancer." (John A. Sampson, in a symposium on misplaced endometrial tissue at the fiftieth annual meeting of the American Gynecological Society, 1925).—ISS.

Endometrioma of the Anterior Abdominal Wall

CHERYL P. FRYDMAN, M.D., JUDITH W. SCHWARTZ, M.D., AND IRA S. SCHWARTZ, M.D.

Abstract

Endometriosis as a tumor of the abdominal wall has been infrequently reported. Twenty-five cases found in the literature occurred in patients who previously had cesarean sections. The patient reported on here had preoperative sonographic and computed tomographic studies. The clinical and pathological findings as well as the proposed mechanisms for endometriosis are reviewed.

Case Report. A 41-year-old white woman, gravida 1, para 1001, was referred to the Mount Sinai Hospital for evaluation of an abdominal mass after consulting her private physician for a one-year history of constant abdominal pain localized to the left infraumbilical region. She reported no associated gastrointestinal or genitourinary complaints. Barium enema and intravenous pyelogram findings were within normal limits. Abdominal ultrasound and CT scan revealed the presence of a 7-cm intraabdominal mass, considered to represent a desmoid tumor (Figs. 1, 2). The patient had two previous uterine myomectomies and had had a cesarean section several years before. Examination on admission revealed an afebrile, well-developed, well-nourished white woman in no acute distress. Her blood pressure was 140/90, her pulse 110/min. Abdominal examination confirmed the presence of a firm, nontender abdominal mass located in the left infraumbilical region. On pelvic examination, a slightly enlarged fibroid uterus without adnexal masses was noted. Routine laboratory examinations were within normal limits.

Excision of the abdominal mass was performed on the first hospital day. Intraoperative evaluation of the abdomen and pelvis revealed a small fibroid uterus and apparent endometriosis of the uterosacral ligaments. The postoperative course was unremarkable.

Pathologic Findings. The excised mass measured 7.0 × 7.0 × 4.0 cm. It consisted of a roughly ovoid, well-circumscribed portion of firm fibrofatty tissue. Section revealed multiple cystic spaces containing red-brown soft material within dense fibrotic tissue, surrounded by yellow-tan fat and strands of skeletal muscle (Fig. 3).

Histologically, the mass consisted of islands of endometrial tissue made up of glands lined by columnar and cuboidal epithelial cells surrounded by endometrial stroma. Some of the glands were dilated. The glands and stroma were dispersed within a background of extensive fibrosis containing chronic inflammatory cells and scattered hemosiderin-laden macrophages (Fig. 4).

Discussion. Endometriosis is defined as the presence of nonneoplastic endometrial elements (glands and stroma) in ectopic sites. It most commonly occurs in the ovaries but may also involve other regions and organs, including the pelvic peritoneum, uterosacral or round ligaments, fal-

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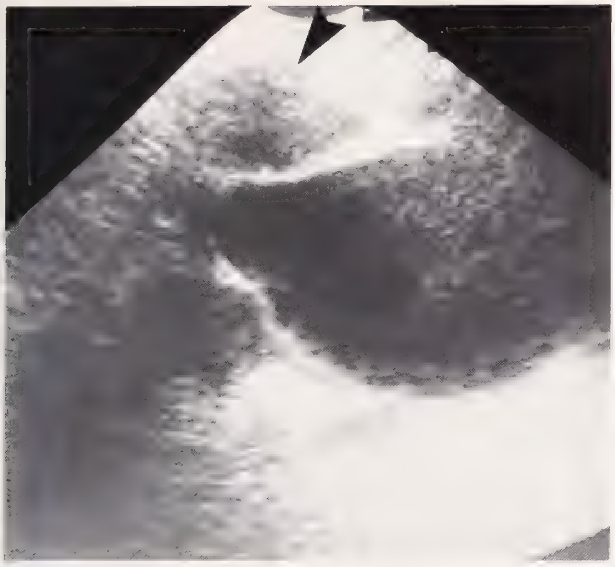

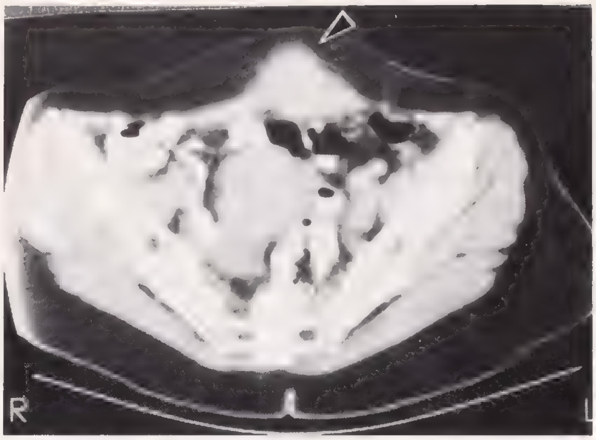

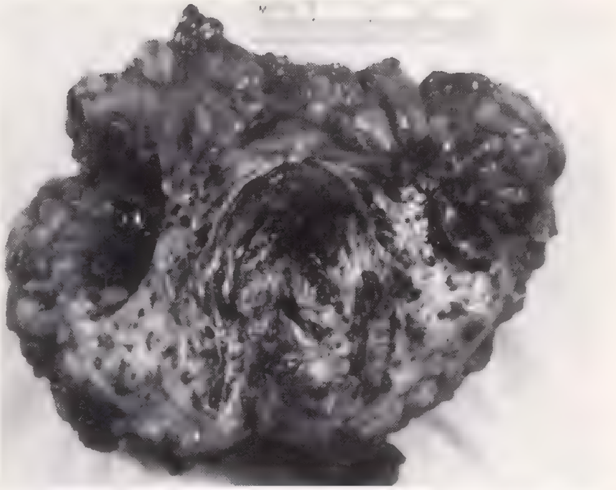

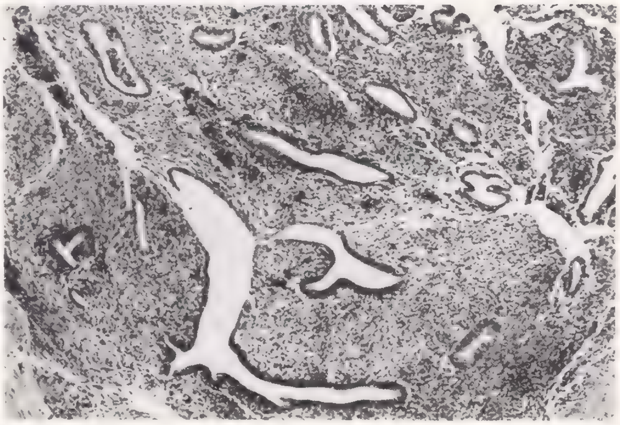

Fig. 1. Top left. Ultrasound. Left parasagittal longitudinal real-time scan shows poorly echogenic mass in abdominal wall anterior to dome of bladder.

Fig. 2. Top right. Computed tomogram at midpelvic level. Soft tissue mass density in anterior abdominal wall, approximately one cm under skin within rectus muscle.

Fig. 3. Middle right. Gross appearance of sectioned mass shows multiple dark cystic spaces in fibrous background bordered by fat and muscle.

Fig. 4. Bottom right. Histology of endometriosis: irregular glands within dense spindle-cell stroma and collections of inflammatory cells (hematoxylin and eosin stain, original magnification, ×100).

lopian tubes, cervix, vagina, vulva, rectum, and sigmoid colon. Less common sites include the urinary bladder, umbilicus, skin, lymph nodes, pleura, lungs, and incisional scars (1). Patients frequently experience dysmenorrhea, dyspareunia, menorrhagia and metrorrhagia, rectal pain, and tenesmus. The clinical diagnosis often rests on the relationship of the symptoms to the menstrual cycle, although many cases are confirmed at laparotomy or laparoscopy by tissue sampling. Ultrasound detection of abdominal wall endometriosis has been documented (2); however, in a recent report multiple ultrasound examinations were negative whereas CT findings were positive (3). The histologic diagnosis of endometriosis requires the presence of two of the following phenomena: endometrial glands, stroma, and deposits of hemosiderin pigment (4).

Surgical scars are one of the most frequently overlooked sites of endometriosis. Approximately fifty cases of perineal endometriosis in an episiotomy or other surgical scar have been reported in the literature (5–9). Steck and Helwig have reported 77 cases of endometriosis occurring in scar tissue, most of which were related to gynecologic procedures. Twenty-five of fifty-six cases involved surgical scars following cesarean sections (10). Three cases of endometriosis in abdominal-wall scar tissue have recently been reported (11). These occurred in women who had undergone cesarean section from three months to three years prior to the onset of symptoms. The latter occurred or were exacerbated during menses and remitted during subsequent pregnancies, reappearing postpartum.

Several theories have been proposed concerning the histogenesis of endometriosis. According to Sampson's implantation theory, the process arises via regurgitation of menstrual endometrium through the fallopian tubes to the peritoneum, where it is implanted (12). This may account for the common involvement of peritoneal surfaces, such as the ovary. Although retrograde menstruation has been amply documented, the endometrial tissue is probably nonviable. The celomic metaplasia theory of Meyer proposes that peritoneal mesothelial cells show müllerian metaplasia of endometrial type under the effect of unknown stimuli, which may include trauma or nonspecific irritation (13). This explains the anatomic distribution of endometriosis to some extent but not fully; the process may also be found in the pleura and pericardium. According to Halban's metastasis theory, endometrial tissue may be disseminated through the lymphatic system (14). This theory provides an explanation for the presence of endometrial foci in lymph nodes or lymphatics of the broad ligament. Hematologic spread of endometrial tissue may account for involvement of the lungs and extremities (15).

It has been suggested that the presence of endometrial tissue in a surgical scar following cesarean section, as seen in the case presented here, may be secondary to direct transplantation at the time of surgery or may alternatively be the result of metaplasia of peritoneal fragments included in the scar during the procedure (10).

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Lita Annenberg Hazen Award 1985

Comments

James F. Glenn, M.D.
President
The Mount Sinai Medical Center

In a span of just five short years, the Lita Annenberg Hazen Award for Clinical Research in Medicine has taken its place as one of the most prestigious of the many recognitions of excellence in the medical sciences. In 1985, two previous corecipients of the Hazen Award, Dr. Joseph Goldstein and Dr. Michael Brown, were further honored with a jointly awarded Nobel Prize. We take great pride in this, since Mount Sinai and the Hazen Award Committee were the first to publicly recognize the outstanding contributions of these distinguished clinical scientists. We fully anticipate that other Hazen Award winners will be similarly recognized in the future.

The Hazen Award is unique and timely. In 1985, the recipient was Hugh O'Neill McDevitt, a pioneer in uncovering the relationship between the genetic control of the immune response and susceptibility or resistance to a wide range of diseases. At the awards ceremony on November 5, 1985, Dr. McDevitt was introduced by Dr. Baruj Benacerraf, 1980 Nobel laureate who is the Fabyan Professor of Comparative Pathology and chairman of the Department of Pathology at Harvard Medical School. The remarks which follow here by Dr. Benacerraf are pointed and revealing, emphasizing the diminishing emphasis placed upon clinical research and the decreasing support for this vital aspect of biomedical science. The Hazen Award encourages investigators and their proteges in the pursuit of excellence in clinical research. Let us also hope that the Hazen Award will stimulate the interest of other private and public sources of funding for the critical area of clinical investigation.

Introductory Remarks

Baruj Benacerraf, M.D.
Director
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Hugh McDevitt, the distinguished recipient of the 1985 Lita Annenberg Hazen Award for Excellence in Clinical Research, ranks among the best in a small group of basic investigators whose contributions have had profound significance for clinical medicine. Hugh McDevitt's career since his graduation from Harvard Medical School exemplifies how a brilliant physician-scientist can elucidate important problems basic to medicine and develop a sound scientific approach to clinical problems, in the best tradition of Flexner's biomedical revolution.

In agreement with the basic aims of the Hazen Award, I must here voice a plea for the training, support, and encouragement of scientifically trained clinicians, at a time when—unfortunately—they are becoming an endangered species.

Hugh McDevitt began his career in the laboratory of Harvard's most distinguished immunologist, Albert Coons, who, as everyone knows, developed the now-classical fluorescent antibody technique and used it to analyze the cellular basis of antibody synthesis. After this valuable initial technical training, McDevitt, demonstrating again a keen judgement for excellence in his choice of teachers, joined John Humphrey's exciting laboratory at Mill Hill in London. John is one of the most charismatic immunologists of this century who inspired and encouraged scores of young scientists in England and abroad with his infectious enthusiasm and his broad curiosity. Working with Michael Sela's synthetic branched aminoacid copolymers, Humphrey had made the fascinating observation that random-bred rabbit strains differed in their immunologic responsiveness to these antigens. McDevitt was fascinated by this finding but perceived correctly that a successful genetic analysis of the phenomenon, which we had also independently observed in guinea pigs, required the use of inbred mouse strains. Accordingly, in 1965, McDevitt was able to report the first demonstration of Ir gene control of antibody responses in the mouse. From then on, realizing the considerable potential significance of this finding, McDevitt systematically pursued the study of Ir gene phenomena until, many years later, it had revealed all its mysterious intricacies.

I met Hugh McDevitt for the first time in Humphrey's laboratory when the early work was in progress. Our common interests, his buoyant and warm personality, his keen intelligence, and his generous and idealistic nature were the basis of a lasting friendship, which made our common study of the genetic control of immunity deeply rewarding.

Soon, McDevitt was appointed assistant professor of medicine at Stanford University School of Medicine, where he has remained ever since, rising to national and international renown as one of the most distinguished scientists of that famous university.

Ir Gene Function. Advances in the analysis of Ir gene function were greatly fostered by the availability of inbred mouse strains and by the fact that these strains had been developed by transplantation biologists and geneticists. Examination by McDevitt of the responder and nonresponder strains to Sela's copolymers revealed that responsiveness could be predicted on the basis of the H-2 haplotype of the strain. Keenly aware, therefore, of the potential contribution of transplantation genetics to the elucidation of Ir gene phenomenology, McDevitt, using H-2 congenic strains, demonstrated the linkage of Ir genes to H-2. Then, with the use of H-2 recombinant strains, he mapped the relevant genes to a new region, the I region of the murine MHC. These fundamental genetic contributions of McDevitt permitted the subsequent analysis by many laboratories of the critical role of MHC molecules in the recognition of foreign antigens by T cells. After his discovery of the I region of H-2, McDevitt took an important part in the identification of the Ia molecules, the class II MHC molecules coded for by the I region, with appropriate alloantisera. These reagents permitted a molecular and genetic analysis of the Ia molecules essential for T-cell immune responses, and for the recognition of self and nonself. More recently, McDevitt, alert to the powerful analytic potential of molecular genetics for studies of MHC molecules, became an expert in these modern techniques and contributed significantly to our detailed knowledge of class II MHC genes.

Clinical Ramifications. While carrying out these seminal studies on the murine system, McDevitt never lost sight of the potential significance of his discoveries for clinical medicine. He was indeed among the first to identify the linkage of a variety of diseases with HLA, the human MHC, and more particularly with the DR region, equivalent to the murine I region, and to stress the significance of this linkage.

In another series of important experiments, McDevitt made practical use of the importance of class II MHC molecules in the associative recognition of foreign antigens by T cells. He demonstrated that the administration of anti-Ia monoclonal antibodies could function as a selective and otherwise harmless immunosuppressive agent, and suppress the development of autoimmune diseases in selected murine strains, thereby opening new therapeutic approaches for these major diseases.

McDevitt has also exerted considerable influence on younger generations of scientists from many countries who have trained in his laboratory and who have carried the impact of his teaching abroad.

Need for Training in Science. A few thoughts on the broader significance of the award of the Lita Annenberg Hazen prize to Hugh McDevitt are in order here. This award is wisely meant to foster research of the highest quality directed to clinical problems, with an emphasis also on the training of young scientists. Hugh McDevitt is a laureate who best exemplifies the prize's intent to encourage the development of clinician-scientists and to publicize and reward their contributions to better health. The dramatic advances of the last fifty years in our understanding, diagnosis, and treatment of disease, which has meant a marked increase in lifespan for all, a decrease in mortality and morbidity in younger age groups, and on the whole a more secure life medically, is the result of the development of scientific medicine, of the training of physicians as scientists as well as clinicians, and of the dedication of a large fraction of our young graduates of medical school to a career in investigative medicine.

I regret, however, that in the last few years, fewer physicians are being attracted to science and investigative medicine, in part for economic reasons, and in part because they perceive that the long-term government support of biomedical research is being compromised. Moreover, I am also concerned that the medical schools have changed their priorities and their curricula to reflect these trends, emphasis being increasingly placed on problems of delivery of care and relative costs. As a consequence, future generations of physicians may lack an appreciation of the sciences basic to medicine. These new concerns, together with the generation of highly sophisticated new teaching schemes, must never replace a sound scientific education for physicians, which is the only training that will endow them with the capacity to develop mature clinical judgement. I also fear that these changes in emphasis in our schools, joined to diminished support for medical research, may compromise the recruitment and training of the clinician-scientists who will provide us, in the future, with the type of seminal contributions that Hugh McDevitt has made and continues to make.

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Immune Genes Introduction

The extraordinary variability of the combining site of the immune receptor is related to the organization of genes encoding variable region of two chains which compose the antigen receptor of lymphocytes.

The major characteristic of these genes is that they are created in mature lymphocytes by rearrangements which join distinct gene segments leading to a single transcript and polypeptide chain, a hallmark property of the lymphocytes rarely observed in other somatic cells.

There is a major difference between the antigen receptor of T and B cells; whereas B cells recognize the antigen alone, T cells can recognize these antigens only in association with products encoded by the genes of the major histocompatibility locus. These products, called class I and class II antigens, are used as genetic restriction elements by T cells to recognize the foreign antigens.

The polymorphism of class I and class II antigens is related to the allelism of genes of the major histocompatibility locus encoding their specificity.

A symposium on immune genes held on April 3, 1985 at Mount Sinai in New York brought together experts in molecular immunology who have devoted their labor and thoughts to understanding the regulation of genes encoding the specificity of T and B lymphocyte receptors and of MHC encoded antigens. The distinguished participants have fashioned the papers published here from the symposium presentations.

In addition to gene regulation, the participants also discuss important aspects of molecular immunology related to the genetic mechanisms responsible for the generation of antibody diversity and of the expression of regulatory idiotopes, which represent targets of regulatory forces and of communication between clones within the immune system.

Particular attention is also given to extraordinary technical achievements which permit us to understand the regulation of immune genes. One of these achievements is the creation of hybrid molecules by transfection or by insertion of genes into the germinal cells—what are now known as "transgenic" mice, the term coined by Gordon and Ruddle.

As chairman of the symposium and guest editor of this resultant issue, I am particularly grateful to Dr. James Glenn, president of The Mount Sinai Medical Center, Dr. Nathan Kase, dean of the Mount Sinai School of Medicine, and Dr. Samuel Elster, dean of the Page and William Black Post-Graduate School of Medicine, for their enthusiastic scientific, financial, and practical support in the organization of the meeting.

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Regulation of Immunoglobulin Variable-Region Gene Assembly

FREDERICK W. ALT, PH.D., MICHAEL G. RETH, PH.D., T. KEITH BLACKWELL, PH.D., AND GEORGE D. YANCOPOULOS, PH.D.

The variable regions of immunoglobulin heavy and light chains are encoded by multiple germline DNA elements that are assembled into complete variable-region genes in precursor (pre-B) lymphocytes. Various analyses have indicated that these processes of gene assembly are highly regulated, but, primarily because of the lack of appropriate models, little information has been obtained on the molecular elements that mediate this regulation. Recently, cell lines have become available which undergo all of these gene assembly events in culture, and studies with these cell lines have yielded some insights into this complex regulatory process. We present here a general model for the regulation of these recombination processes and discuss the evidence for this model.

Assembly and Expression of Immunoglobulin Genes

Analyses of tumor cell lines and sorted cell populations that represent early and late pre-B cells have suggested that during B-cell differentiation the assembly and expression of heavy chains precedes that of light chains (1). The variable region of the immunoglobulin heavy chain is encoded by three separate germline DNA elements: the variable (V_H), diversity (D), and joining (J_H) segments (2). These segments are assembled during the earliest known stage of B-cell differentiation (pre-B cells) in an ordered sequence: joining of D to J_H occurs first and on both chromosomes, followed by joining of a V_H segment to the DJ_H complex (3). Developing B-lymphoid cells which form a productive (in phase) V_HDJ_H rearrangement express a cytoplasmic μ heavy chain that generates the cytoplasmic μ-positive, light-chain-negative pre-B cell. After the production of a heavy chain, pre-B cells begin the process of assembling the genes for a light chain. Light chains can be of either the kappa or the lambda type; these types are encoded by separate gene families, which reside on different chromosomes (2). For either type of light chain, assembly of variable-region genes involves the fusion of two V-gene elements, V_L and J_L (2). Productive assembly of a gene for the variable region of a light chain leads to the production of a light-chain protein; if this protein can functionally interact with the preexisting cellular heavy chain, the complex is deposited on the surface of the cell as an immunoglobulin receptor (4).

A Model of B-Lymphocyte Differentiation

The Abelson murine leukemia virus (A-MuLV) is a replication-defective retrovirus that is unique in its capacity to transform immature B-lymphoid cells in culture (5). Studies of the early stages of B-lymphocyte differentiation have been facilitated by the availability of cell lines transformed by A-MuLV; much of the evidence in support of the ordered rearrangement pathway outlined above has been obtained from studies of such cell lines (3, 6). A-MuLV transformants derived from fetal liver, the site of B-cell differentiation in the fetal mouse, are usually null cells with DJ_H rearrangements at both heavy-chain alleles and no light-chain rearrangements. When propagated in culture, such lines often append V_H gene segments to the preexisting DJ_H complexes, allowing dynamic studies of the process of V_H

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gene assembly. For example, analyses of the V_H segments used to form V_HDJ_H complexes in such lines have indicated an extreme bias for use of the most 3′ known V_H gene segment (V_H81X) (7); a similar bias in V_H gene usage was subsequently observed in normal populations of pre-B cells (8). A-MuLV transformants derived from adult marrow, the site of B-cell differentiation in the adult mouse, have generally assembled at least one V_HDJ_H at the time of isolation, and many produce cytoplasmic μ heavy chains (6); a few such lines have also assembled light chains at the time of isolation or assemble kappa light chain genes when propagated in culture (6, 9). Recently, we characterized A-MuLV transformants that start with two DJ_H rearrangements and no light-chain gene rearrangements, but which sequentially assemble genes for the variable region of a heavy chain and then genes for the variable region of a kappa light chain during growth in culture. Ultimately, these lines generate subclones that express complete immunoglobulin on their surface (4), a phenotype associated with the B lymphocyte. Thus, such lines undergo all of the sequential events in gene rearrangement and expression associated with the pre-B-lymphocyte stage of B-cell differentiation.

Ordered Rearrangements and Allelic Exclusion. Although a given B-cell clone has the potential to assemble and express two totally different variable-region genes at each heavy and light chain "allele" and, by extension, make immunoglobulin receptors with multiple specificities, each B-lymphocyte clone produces a monospecific immunoglobulin receptor. This monospecificity is a result of the phenomenon of allelic exclusion (10); a single B-cell clone expresses just one of its two heavy-chain alleles and one of its several light-chain alleles (two for kappa and at least six for lambda) as immunoglobulin chains which combine to form surface immunoglobulin. Evidence from the study of permanent cell lines indicated that heavy-chain allelic exclusion results from shutdown of the process of V_H-to-DJ_H joining upon the production of a complete heavy-chain protein (3, 6); similarly, light-chain allelic exclusion seems to result from shutdown of V_L-to-J_L joining once a light chain is produced which can combine with the preexisting cellular heavy chain to form a complete immunoglobulin molecule (11). Recently, studies of transgenic mice (10) and studies of A-MuLV transformants which immunodifferentiate in culture have provided direct support for these models. Studies of A-MuLV transformants that had two DJ_H alleles showed that in progeny that have assembled a productive (in phase) V_HDJ_H rearrangement, the rate of V_H-to-DJ_H joining on the remaining DJ_H allele was at least greatly diminished (MGR and FWA, (unpublished study).

Two general types of models have been proposed to explain the general occurrence of heavy-chain gene assembly before light-chain gene assembly: stochastic models, which suggest a greater probability of heavy-chain rearrangement than of light-chain rearrangement (12) and models which predict sequential differentiation phases for heavy and then light chain gene assembly (6). According to the stochastic models, there should be some cells which form productive light chain rearrangements before forming a productive heavy chain rearrangement. We looked for such cells by analyzing subclones of an A-MuLV transformant that assembles both heavy and light chain genes during propagation in culture (4). Among the hundreds of subclones we have analyzed, we have yet to find a line that undergoes light chain rearrangement without having assembled and expressed a complete heavy chain gene. Furthermore, all subclones which did express complete heavy chain genes began to assemble kappa light chain genes. No subclones with lambda gene rearrangements were observed, consistent with the idea that assembly of kappa genes precedes that of lambda genes. These findings strongly support the model of separate phases of assembly of heavy and light chain genes. Furthermore, these results indicate a dual regulatory function for μ heavy chains: signaling the cessation of V_H assembly and signaling the onset of V_L assembly.

Control of Assembly of Variable-Region Genes. Regulation of the assembly of immunoglobulin V-gene segments is manifested at several levels, including the orderly assembly of J_H, D, and V_H gene segments; the orderly assembly of V_H, V_κ, and V_λ genes; and the allelic and isotype exclusion of V_H and V_L gene segments. The recent discovery that V-gene segments and their flanking recognition sequences in T-cell receptors are quite similar to V_H and V_L gene segments (13) suggests that "V" gene assembly may be regulated in a tissue-specific manner as well. Various mechanisms can be proposed for the tissue- and stage-specific assembly of this set of related recombination events. However, several lines of evidence point to one general type of mechanism:

1. The recombination recognition sequences (heptamers and nonamers) flanking the unrearranged gene segments used to assemble the variable regions of heavy and light immunoglobulin chains and the V genes of α, β, and γ T-cell-re-

ceptor chains are all quite similar (13). This suggests that they might all be recognized by the same recombinase.

2. The unrearranged V_H gene segments are transcribed and nuclease-sensitive in cells undergoing rearrangement of V_H to DJ_H, but are neither transcribed nor sensitive in more mature cells which express a complete heavy chain (14). Furthermore, induction of heavy chain synthesis is correlated with the cessation of transcription of germline V_H genes. This is consistent with the idea that the μ heavy chain leads to the cessation of V_H-to-DJ_H joining by "closing down" the V_H region and making it inaccessible to recombinase.

3. Pre-B cells that are producing heavy chains and actively rearranging V_L segments do not undergo V_H-to-DJ_H joining but do form nonproductive, yet site-specific, rearrangements of non-V_H sequences to unrearranged J_H segments (GDY et al, unpublished study). This result is consistent with the idea that the same recombinase rearranges V_H and V_L segments, but that in μ-positive pre-B cells the μ protein signals not only the closing of the V_H region but also the opening of the V_L (J_L) region—allowing V_L assembly by the same recombinase that was formerly assembling V_H genes. Site-specific rearrangements of non-V_H sequences can occur because, although the V_H region is closed, the J_H region and perhaps other nearby regions containing recombination sequences are still open.

4. The J_H region is transcribed and nuclease-sensitive in many T cells, which are capable of undergoing D-to-J_H joining. However, the V_H gene segments are not transcribed in T cells, which never make V_H-to-DJ_H joins (GDY et al, unpublished study; (15,16). This is consistent with the idea that the same recombinase assembles immunoglobulin V genes and T-cell-receptor V genes; a complete V_H gene is not assembled in T cells because the V_H gene segments are not accessible in these cells.

Together, these observations suggest that both the assembly of immunoglobulin V-gene segments and the assembly of T-cell-receptor V-gene segments are mediated by a single recombinase and that control of specific recombination events is mediated by the accessibility of the individual substrate gene segments to this common recombinase.

Gene Transfer Studies of Assembly of V Genes. We have previously demonstrated that molecularly cloned D and J_H segments undergo site-specific recombination when introduced into the genome of an A-MuLV transformant that is actively assembling its endogenous V_H gene segments (8, 17). Several predictions based on the regulatory model outlined here are testable by this type of gene transfer technology. One such testable prediction is that in a line that has high levels of endogenous recombinase activity, the capability of V-gene segments to be assembled should be correlated with their "openness." To test this prediction we designed a recombination substrate which contained a germline D segment and the four J_H segments upstream of the herpesvirus thymidine kinase (TK) gene. The construct was introduced into a TK^- A-MuLV transformant, which actively rearranged its endogenous V_H gene segments. As observed previously, in most single-copy transformants the introduced TK gene was inactive; significantly, these cells showed no evidence of D-to-J_H rearrangement within the integrated construct (20). However, subclones selected for growth in medium containing HAT, in which the introduced TK gene was activated, showed a high level of D-to-J_H joining within the integrated construct. Thus, despite high levels of endogenous recombinase activity, joining of D and J_H segments is dependent on the activity of a nearby gene, perhaps mediated through a local alteration of chromatin structure.

A second prediction of the regulatory model is that all V-gene segments—of both heavy and light immunoglobulin chains and α and β T-cell-receptor chains—should be rearranged in any cell type having high levels of recombinase (regardless of which endogenous genes are being rearranged) if they are present in an open configuration. As a preliminary test of this prediction we flanked the herpesvirus TK gene with T-cell-receptor D_β and J_β segments, introduced this construct into an A-MuLV transformant that actively assembled V_H genes but not T-cell-receptor V genes, and selected for site-specific recombinants as described previously (20). Preliminary results indicate that, within such an open construct, the T-cell-receptor V-gene segments are assembled at high frequency by B cells.

Thus, together, these gene transfer experiments provide strong support for the model in which tissue- and stage-specific assembly of the various V gene segments is mediated by modulation of the accessibility of substrate gene segments to a common recombinase.

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The Igh-V Gene Repertoire of the Mouse

Roy Riblet and Peter H. Brodeur

One of the central questions in immunology over the last several decades has been how antibody diversity is generated, that is, how do the B cells generate a wide variety of structures for the antibody combining site? Our knowledge of the structure of the genes that code for antibodies tells us a great deal about antibody diversity (1): Immunoglobulin genes come in pieces, as do most eukaryotic genes, but the complexity of the organization is more extensive than the standard intron-exon arrangement of most eukaryotic genes, in that the pieces or the gene segments come in clusters, and a mature gene is assembled by choosing one element from each cluster.

For light chains, prior to gene rearrangement and assembly, there is an array of V segments, one or more joining segments, and one or more constant-region gene segments. In an antibody-producing B cell, a V gene becomes joined to a J segment, with deletion of the intervening DNA, to form the transcribed gene.

The heavy-chain gene organization is similar but more complex. Again there is an array of V segments. There is an additional set of genetic elements for heavy-chain genes called D segments, and again J segments and C segments. Joining a Vh to a Dh to a Jh creates a complete heavy-chain V region gene. This process provides a great deal of diversity because, for light chains, any V can be joined with any J, and for heavy chains, any V can be joined with any D and with any J. This combinatorial aspect generates a wide range of antibody variable-region sequences and combining site structures.

The assembly process is not free of errors. In fact, it is error-prone. Each of these gene-splicing acts is prone to errors and can generate new information—DNA sequences that did not exist in the germline but now do exist in a particular B cell clone. These joining errors further amplify the diversity created by the combinatorial joining process.

Even after the gene is assembled in a mature and active state, additional diversification occurs through the ongoing process of somatic mutation, which continues throughout the life of the B cell clone. Mutations accumulate in the V gene, further modifying the structure of the combining site and the specificity of the antibody (2).

The mechanisms of somatic diversification—combinatorial joining, joining errors, and somatic mutation—greatly amplify the diversity present in the germline, in the set of inherited gene segments. But the inherited set of gene segments is the starting point for these processes, and its composition can be surmised to have a crucial role in determining the eventual repertoire and the way in which the individual deals with its environment. To begin to assess these issues, we address here a series of questions to examine specifically the germline library of heavy-chain variable-region segments: How many Vh genes are there? What are their structures? What are the specificities of the antibody proteins they encode? What is their physical arrangement, and does it have any functional consequence? How flexible are the evolutionary constraints on this germline library of Vh genes—that is, are there constraints on the structure of the library, or is there a great deal of flexibility?

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TABLE 1
Mouse Igh-V Gene Families

Vh Family	3609	X24	J606	36–60	J558	S107	Q52N	7183	VGam3-8
Complexity	12	2	10	5	60	4	15	12	5
CDR2 Length	16	17	19	16	17	19	16	17	17
Antibody Specificities									
Flu HA	–	–	–	+	+	+	+	+	–
Lysozyme	–	+	–	+	+	–	+	+	–
DNA	–	–	–	–	+	+	+	+	–
GAT	–	–	–	+	+	–	+	+	–
Digoxin	–	+	–	+ + +	+	–	–	+	–

The Inherited Library of Vh Gene Segments. An approach to these questions is to use cloned Vh genes to analyze the mouse genome by Southern blots. When this is done, each Vh gene probe hybridizes to a series of EcoRI fragments, each fragment carrying a Vh gene related to the probe. Using more than 30 Vh genes for this analysis revealed only eight patterns of hybridizing fragment (3, 4). These patterns define eight families of related Vh gene segments (Table 1). Recently, a ninth pattern and family have been identified (5).

The families range from very small (the X24 family has only two Vh genes) to very large (the 558 family has at least 60 members). The total number of hybridizing fragments on an EcoRI digest is about 120, that is, 120 heavy-chain variable-region genes in a BALB/c mouse. These Vh gene families include all the genes that code for all known antibody protein sequences in mice (6). A recent study of LPS hybridomas suggests that these 9 Vh gene families comprise the entire inherited repertoire (7).

Why Are There Different Vh Gene Families? We have surveyed a wide variety of wild mice and closely related species, and all have comparable germline repertoires; every Vh gene family is present, although with some variation in size. At least some of these Vh gene families are represented in the human genome and even in crocodiles. We can begin to ask what the point of this organization might be. Examination of Vh gene sequences reveals that the families differ in the length of CDR2, the second complementarity-determining region (8), that is, the second portion of the Vh sequence that loops out to form the end of the molecule and the antibody combining site. The heavy chains encoded in three families—Q52N, 3609 and 36-60—have a second CDR length of 16 amino acids. Four families have a second CDR length of 17 amino acids, and two have a second CDR length of 19 amino acids (Table 1).

These length differences are consistent for the available sequences within a family, and one can speculate that they have some meaning—that they create different possibilities for the shape or the nature of the combining site. If that is true, we might expect to see some bias or specialization in the way that these families are used in making particular types of antibodies. Unfortunately, the available data do not reveal any kind of bias at all. Antibodies against bacterial carbohydrates can be found coded by genes in every one of the V gene families. Antibodies against influenza hemagglutinins are coded by five of the nine families (Table 1). Anti-lysozyme, anti-DNA, anti-GAT, and anti-digoxin sequences also derive from four or five different Vh gene families, and it seems likely that continued searching will identify examples of these specificities coded by Vh genes in yet more families.

What this says is that simplistic speculation about specialization of Vh gene families is not supported. Rather, we have evidence of a great deal of flexibility in the way these genes are used. Nonetheless, a more acute analysis of structure and specificity should reveal some relationships that can be surmised to be the evolutionary reason for the continued presence of these different structural themes.

Genetic Polymorphism of the Germline Repertoire. Another aspect of the flexibility of the antibody gene repertoire is seen in a Southern blot showing the VhJ558 gene family in 18 mouse strains (Fig.). The strains are organized according to their allotypes; the first three strains have the *a* allotypes, the *a* alleles of the heavy-chain constant-region loci (Igh-C genes). The next two strains have the *b* allotypes and the seventh and eighth strains have *c* allotypes, and so on. Examination of this strain survey demon-

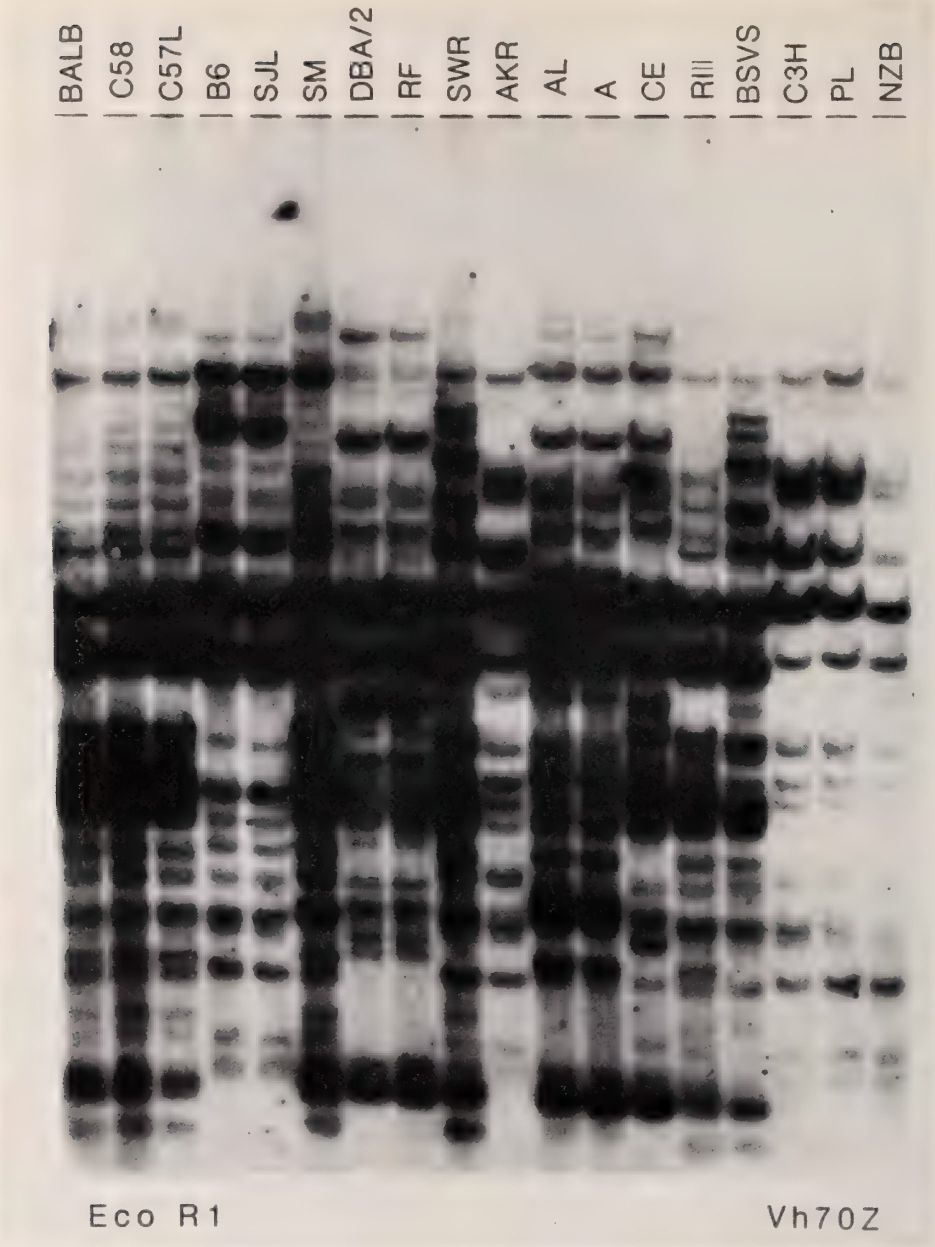

Fig. Southern blot of VhJ558 gene family in eighteen inbred mouse strains. Liver DNA from listed mouse strains was digested with EcoRI, electrophoresed in an agarose gel, and blotted onto a nitrocellulose membrane. Blot was hybridized with a nick translated plasmid containing the actively rearranged Vh gene from the 70Z B cell lymphoma; this Vh gene is a member of the J558 Vh gene family (3).

strates a clear correlation; strains having the same allotype also have the same pattern of Vh genes. That is true for each of the Vh gene families, so that among mice there are a large number of "starting sets" of heavy-chain genes, each set differing throughout from the others.

This figure, in fact, shows only genetic polymorphism of the restriction fragments carrying the Vh genes, but in those cases in which Vh genes have been cloned from different strains, there are nucleotide substitutions within the coding region that correlate with the differences in restriction fragment sizes. Thus, polymorphism on a Southern blot is reflective of polymorphism at the sequence level. So again it seems that the library of Vh genes has a great deal of flexibility, (allotypically) different mouse strains have approximately the same overall number of Vh genes distributed among the same families, but many, perhaps all, individual genes differ be-

TABLE 2

Mapping Vh Gene Families with Igh Recombinant Strains

Strain	VhJ558		VhS107		VhQ52N		Vh7183		Igh-C
BALB/c	a		a		a		a		a
A/He	e		e		e		e		e
AXC-2	a	×	e		e		e		e
AXC-9	e	×	a		a		a		a
AXC-7	e		e × a		a		a		a
AXC-8	e		e	×	a		a		a
AXC-1	a		a		a	×	e		e
AXC-4	a		a		a		a	×	e

tween strains. The whole array of Vh (and Ch) genes from an A/J mouse is different from that in a BALB/c mouse and that in a C57BL/6 mouse. This extensive variability suggests that the particular sequences of Vh genes in the array are not as important as are the total number and the diversity of the spectrum of Vh genes. The mechanisms of somatic diversification must have an adequate base of structures to operate on.

Organization of Vh Genes and Families. The polymorphism shown in the Southern blot makes it possible to determine the structure of the array of 120 Vh genes. Our approach is to take two strains with different Vh gene arrays, cross them, and select the rare mice which have a genetic recombination among the Vh genes. We detect recombinants by scoring two serologic markers, the *Igh-1* allotype (the constant-region gene marker) and a particular idiotype (J558) that is inherited and dominantly expressed in the antidextran response. We have created panels of Igh recombinant strains that differ in the portions of the V gene array derived from each parent. DNA is prepared from these strains and analyzed on replicate Southern blots using probes for each Vh gene family (Table 2).

The AXC Igh recombinant strain panel was derived from the BALB/c × A/He cross, selecting recombinants by serology. The J558-antidextran response idiotype marker is coded by a Vh gene in the J558 gene family and the allotype marker by one of the Igh constant region genes in the Igh-C cluster.

The Southern blots show in each AXC strain whether the strain has, for example, the VhS107 family characteristic of BALB/c or A/He. In Table 2 this is summarized as an allele designation, a or e. Thus, AXC-2 strain has the BALB/c type J558 Vh gene family (and antidextran idiotype) but all other Vh gene families and constant-region genes are A/He type. When the data is ordered as in Table 2, a simple genetic map results and the order of the gene families is revealed. Many genetic recombination events (crossover points) fall between Vh gene families but others result in a mixed pattern for a Vh family, for example, the AXC-7 strain has a composite VhS107 gene family, with some Vh genes drawn from BALB/c and some from A/He.

Table 2 presents a partial map of the Vh gene array. When the remaining families, J606, 36-60, X24, and 3609, were analyzed, they were always concordant with the VhJ558 family, indicating that these gene families all lie to the left of J558, and are not ordered by the data available. (The ninth family, VGAM3-8, has not yet been analyzed in this fashion.)

Although we have not completely defined the organization of the Vh gene array, we have used additional mouse genetic tools to estimate the total size of the locus. Southern blot analysis of over 70 recombinant inbred strains of various origins has shown that the total length of the *Igh* locus is 2 centiMorgans or less, that is, 2cM is the 95% upper confidence limit. This is very large for a single locus, but still quite a small piece of a chromosome (chromosome 12 is about 70 cM).

Summary. There are nine Vh families that range in content from 2 to at least 60 members. The total is at least 120. Within a family, genes have a high degree of homology, between 80% and 100% sequence identity. Between families, degrees of homology are much lower, between 50% and 70%. There is a great deal of polymorphism in all Vh families, in Dh, and in C region genes as well, and it is correlated to give Igh haplotypes; that is, the whole 2 centiMorgan region of the chromosome that carries the Vh, Dh, and Ch segments varies coordinately in inbred strains of mice. We were able to map part of this locus and estimate its total length.

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Production of Novel Immunoglobulin Molecules by Gene Transfection

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Antibody molecules and the genes that control their expression have been objects of investigation for several decades. Early studies showed that proteins with the least electrophoretic mobility, the gamma globulins, were specifically removed upon reaction with antigen (1). However, studies of these proteins were hindered by their structural heterogeneity.

With the realization that myeloma proteins represent monoclonal antibodies, great progress was made in the understanding of immunoglobulin (Ig) structure. Structural studies of the myeloma proteins showed that antibody molecules are composed of heavy (H) chains and light (L) chains. Both heavy and light chains contain a variable portion, which is important in determining the specific reaction of an antibody with its antigen, and a constant region, which is important in determining the effector functions of the immunoglobulin molecules. The techniques of molecular biology have permitted detailed analysis of the immunoglobulin genes. These studies have shown that expression of both heavy and light chain genes requires the somatic assembly of DNA segments.

Gene transfection is another technique used in investigating immunoglobulin production. Immunoglobulin genes introduced into nonlymphoid cells are not efficiently expressed (2). However, rearranged kappa light chain genes introduced into either a cell line transformed with Abelson murine leukemia virus (A-MuLV) (3) or a myeloma or hybridoma cell line (4) are efficiently expressed. Similarly, rearranged heavy chain genes introduced into myeloma cells are expressed at high levels (5, 6). Gene transfection techniques have been used to identify specific regulatory sequences within the immunoglobulin genes which are important for their high-level expression (5–11). These regulatory sequences function in a tissue-specific manner and are, at least in part, responsible for the high-level expression of immunoglobulins seen during B-lymphocyte differentiation. Also, since myeloma cells are capable of high-level expression of the transfected genes, it is possible to use gene transfection to produce novel immunoglobulin molecules.

Several methods exist for introducing DNA into cells. Calcium phosphate ($CaPO_4$) precipitation is routinely used to introduce DNA into many types of cells (12–14) and has been used to introduce DNA into lymphoid cells. However, stable transfectants are recovered only at a low frequency from myeloma cells (3, 4) when $CaPO_4$ precipitation is used. Treatment with DEAE-dextran has also been used to introduce DNA into lymphoid cells and in many cases appears to be more efficient than $CaPO_4$ precipitation (7, 8, 10).

The most efficient way to introduce DNA into lymphoid cells may be through protoplast fusion (15). In this method *Escherichia coli* bearing plasmids of interest are treated with lysozyme to remove the bacterial cell walls. The resulting spheroplasts are fused with the myeloma cells by the same methodology used to create somatic cell hybrids. With the protoplast fusion technique, stable transfectants have been isolated at frequencies ranging from 10^{-4} to 10^{-3} (4–6).

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Recent results have suggested that electroporation may rival protoplast fusion as an efficient method for introducing DNA into lymphoid cells (16). In this method a high-voltage pulse is applied to cells in suspension in the presence of DNA. Stable transfectants were isolated from some cell lines at a frequency of 3×10^{-4}. Many different types of cells appear to be efficiently transfected by this method.

Following DNA transfection, either gene expression can be studied transiently or stable transfectants can be selected. To study transient expression, cell populations are harvested at an appropriate interval (usually 40–72 hours) after being transfected with the appropriate DNA. Gene expression is usually monitored by S1 analysis of the messenger RNA produced (17). This approach provides a convenient assay for gene expression, but only small quantities of the product of the transfected gene are obtained, so it is not suitable for the production of novel protein molecules for study.

In order to obtain long-term production of the product of the transfected gene, stable transfectants must be selected. One way to achieve this is to include the gene of interest in a vector which contains a dominant selectable marker—that is, a genetic marker which will produce a selectable change in the phenotype of the normal cells. The most commonly used vectors have been the pSV2 vectors (18–20). These vectors contain the pBR322 *ori* and β-lactamase genes and hence can be propagated and selected as plasmids in *E. coli*. In addition, they contain a transcription unit with a simian virus 40 (SV40) early promoter at its 5′ end and an SV40 segment containing an intervening sequence and polyadenylation signal at its 3′ end. This transcription unit is used to direct the transcription of a selectable gene. Two commonly used selectable genes are *gpt*, the bacterial xanthine-guanine phosphoribosyltransferase gene (19), and *neo*, the phosphotransferase gene from Tn 5 (20). Expression of *gpt* enables mammalian cells to grow in medium containing mycophenolic acid and xanthine (21). Expression of *neo* confers resistance to the antibiotic G418 (22, 23).

Novel Molecules by Chain Assembly

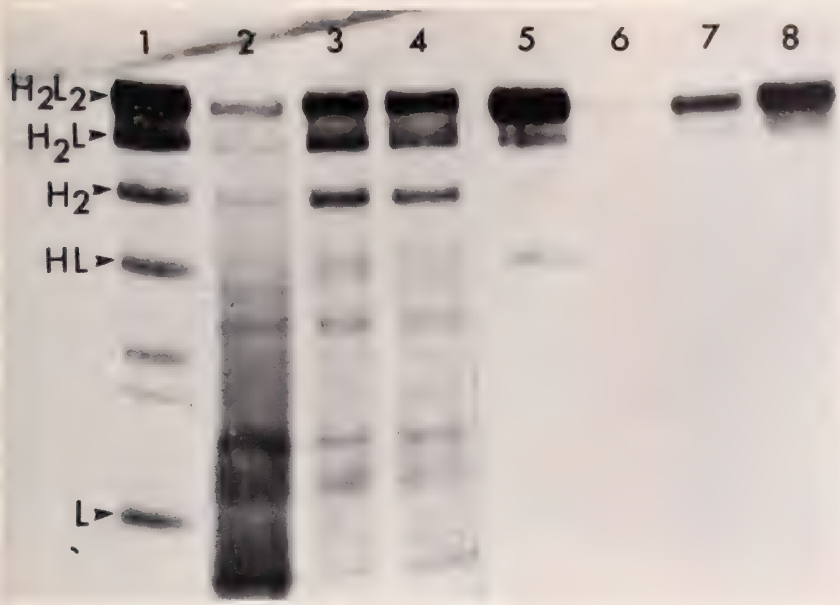
Reassembly of heavy and light chains in vitro has been used to create novel immunoglobulin molecules and to study chain interactions (24–26). However, this method requires that the interchain disulfide bonds be broken and the heavy and light chains separated from each other in the presence of denaturing agents. Although some chain reassembly occurs, the method is inefficient in producing active antibody molecules. A large percentage of the immunoglobulin is not faithfully renatured (25).

Gene transfection provides an alternative means for assembling novel chain combinations. Expression of molecules following gene transfection has the advantage that chain assembly takes place by the normal pathway. The assembled molecules have not been denatured, and hence their structure and function should accurately reflect those of native molecules.

To determine whether novel combinations of immunoglobulin heavy and light chains could be produced by gene transfection, the gene for the γ_{2b} heavy chain of the myeloma MPC-11 was transfected into J558, an immunoglobulin A (IgA), λ myeloma. The transfected heavy chain was efficiently expressed and assembled with the endogenous light chain. Molecules with the compositions H_2, H_2L, and H_2L_2 are clearly visible in the cytoplasm of the transfectants (Fig. 1, lanes 2–4). Much less of the HL composition is present than is seen when the MPC-11 heavy chain assembles with its light chain in the original myeloma (Fig. 1, lane 1). Only the fully assembled H_2L_2 form is seen in the secretions of the transfectants (Fig. 1, lanes 6–8). There is no evidence for the formation of covalent bonds between the γ_{2b} and α chains in either cytoplasmic or secreted immunoglobulins. These novel molecules with the MPC-11 heavy chains joined to λ light chains have been useful in exploring the relative contributions of the variable regions of heavy and light chains to idiotypic determinants (C. Victor-Kobrin, unpublished observations). A mouse IgA heavy chain is also synthesized when it is transfected into a light-chain-producing variant of J558. The transfected heavy chain appears to assemble noncovalently with the endogenous lambda light chain, since immunoprecipitates made by using antiserum against alpha heavy chain contain both alpha and lambda chains (Fig. 2). As is often observed, different transfectants exhibit different levels of expression (compare left and middle lanes in Fig. 2).

Novel Molecules by Modification of Ig Genes In Vitro

The ability to transfer immunoglobulin genes into lymphoid cells, where they produce proteins in sufficient quantities for structural studies, provides an opportunity to produce novel immunoglobulin molecules. By using standard



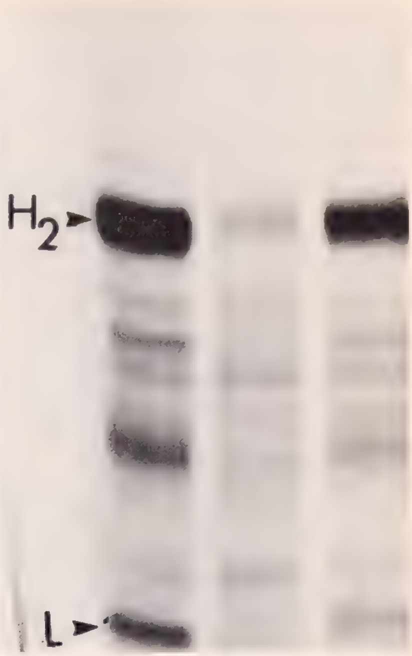


Fig. 1. Left Synthesis, assembly, and secretion of γ_{2b} heavy chain transfected into IgA, λ myeloma J558. Cells were labeled with $[^{14}C]$valine, threonine, and leucine, and cytoplasmic lysates and secretions were prepared. Lanes 1–4 show immunoglobulins precipitated with antiserum against γ_{2b} from cytoplasmic lysates of cells labeled for 30 minutes. Lanes 5–8 show immunoglobulins precipitated from cell secretions after labeling for 3 hours. Lane 1, MPC-11; lane 2, transfectant 1; lane 3, transfectant 2; lane 4, transfectant 3; lane 5, MPC-11; lane 6, transfectant 1; lane 7, transfectant 2; lane 8, transfectant 3. Immunoprecipitates were analyzed on 5% sodium dodecyl sulfate-polyacrylamide gels. Position of bands was determined by autoradiography. Composition of intermediates in assembly is noted at left.

Fig. 2. Right Synthesis of alpha heavy chain transfected into myeloma J558L, which produces a lambda light chain. Transfectants were labeled with $[^{14}C]$valine, threonine, and leucine for 30 minutes, and cytoplasmic lysates were prepared. Immunoglobulins were immunoprecipitated with an antiserum against alpha heavy chain and analyzed on 5% sodium dodecyl sulfate-polyacrylamide gels. Position of migration of heavy chain dimers (H_2) and lambda chain (L) is indicated at left.

recombinant DNA techniques it is possible to attach any variable (V) region to any constant (C) region. It is thus possible to effect isotype switching by transfection techniques. The IgA gene described above was, in fact, made in vitro by splicing the variable region of the heavy chain from an anti-dansyl hybridoma to the constant region of the alpha chain cloned from an unrearranged alpha chain gene (J. Dangyl, unpublished observations). This IgA gene is expressed and leads to the production of a wild-type alpha chain (Fig. 2).

It is also potentially possible to join variable regions from the heavy chains of one species to constant regions from another species, creating cross-species chimeric molecules. It is of particular interest to create molecules in which the variable region is derived from a mouse hybridoma and the constant region is human in origin. These molecules should provide proteins whose specificity is derived from the mouse hybridoma but which exhibit human effector functions. To date, it has been very difficult to produce human hybridomas of the desired specificities. The chimeric human-mouse proteins could fulfill some of the functions of human hybridomas.

To create chimeric human-mouse heavy chains, variable-region genes from either the anti-phosphocholine myeloma S107 or the anti-

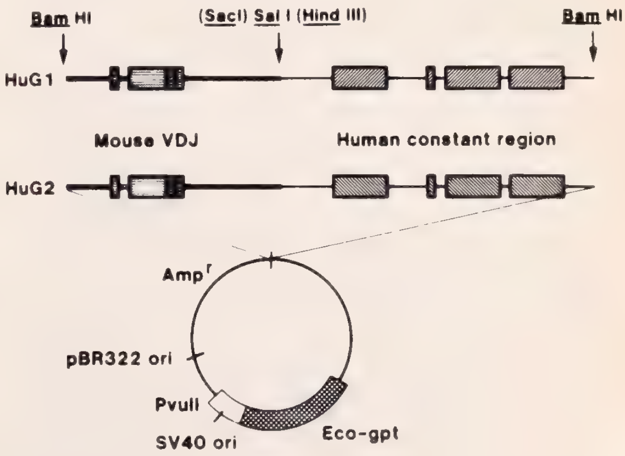


Fig. 3. Schematic diagram of vectors for chimeric mouse-human heavy chain genes. DNA fragments are not to scale. The *Hind* III-*Bam* HI human gamma-1 (HuG1) or gamma-2 (HuG2) heavy chain genes are 7 kilobases. *Hind* III sites in human gamma-1 and gamma-2 heavy chain genes were ligated to *Sac* I site in mouse V_H genes using *Sal* I linkers.

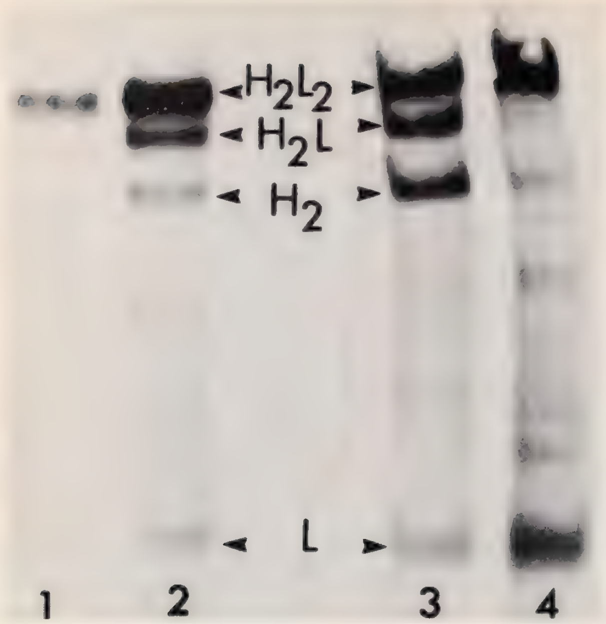


Fig. 4. Left Synthesis of chimeric human-mouse heavy chains transfected in lambda-chain-producing myeloma J558L. Transfectants were labeled with [^{14}C]valine, threonine, and leucine for either 30 minutes or 3 hours. Lanes 2 and 3 show immunoglobulin precipitated from cytoplasmic lysates after labeling for 30 minutes; lanes 1 and 4 show immunoglobulins precipitated from secretions after labeling for 3 hours. Lanes 1 and 2 are from transfectant produced with V_H DNS-HuG1; lanes 3 and 4 from transfectant produced with V_H DNS-HuG2.

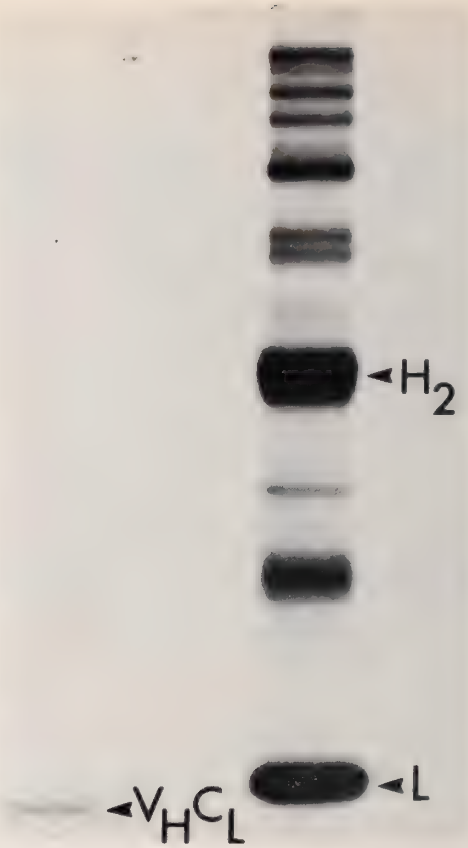


Fig. 5. Right Synthesis of chimeric gene in which variable-region genes from heavy chain of an anti-dansyl hybridoma were joined to constant-region genes from a kappa light chain. Chimeric gene was transfected into nonproducing myeloma. Transfectant was labeled for 30 minutes with [^{14}C]valine, threonine, and leucine, and cytoplasmic lysates were prepared. Chimeric chain was immunoprecipitated with antiserum against kappa light chain. Immunoprecipitates were analyzed on 5% sodium dodecyl sulfate-polyacrylamide gels. Position of migration of V_HC_L chimeric chain is marked. For comparison, immunoglobulin immunoprecipitated from S107 myeloma is shown. Position of migration of light chain (L) and heavy chain dimers (H_2) is indicated.

dansyl hybridoma 27-44 (DNS) were joined to constant-region genes from human gamma-1 or gamma-2 heavy chains by the scheme shown in Fig. 3. The resulting chimeric heavy chains contained in the vector pSV2-gpt were transfected into J558L cells or into cells from a nonproducing myeloma, and stable transfectants were selected with mycophenolic acid. Figure 4 shows the immunoglobulins produced when J558L cells were transfected with chimeric genes representing either DNS variable region and human gamma-2 constant region (lanes 1 and 2) or DNS variable region and human gamma-1 constant region (lanes 3 and 4). The transfected chimeric heavy chains are efficiently expressed. They assemble with the endogenous mouse lambda light chain, and H_2, H_2L, and H_2L_2 molecules are present in the cytoplasm (lanes 2 and 3). Only fully assembled H_2L_2 molecules and free light chain are seen in the secretions (lanes 1 and 4). Similarly, when the variable-region genes from S107 heavy chain are joined to the constant-region genes from human gamma-1 or gamma-2 heavy chain, the chimeric heavy chain is expressed following transfection into either J558L or a nonproducer.

It is also possible to express an immunoglobulin molecule in which both heavy and light chains are chimeric. To that end, the variable region of the S107 myeloma was joined to human kappa chains, and the chimeric gene was placed in the pSV2-neo vector. This vector was then transfected into a cell which was producing the chimeric heavy chain made from S107 variable-region genes and human gamma-2 constant-region genes, and stable transfectants were selected with G418. Some transfectants produced both chimeric heavy and light chain genes. These assembled together and were secreted as H_2L_2 molecules. The H_2L_2 molecules bound antigen (27).

Chimeric molecules can be made in which the variable region from a heavy chain is joined to the constant region from a light chain. Initial studies with the variable region derived from the heavy chain of an anti-azophenylarsonate hybridoma showed that such molecules could assemble with the normal light chain. This heterodimer bound antigen (28). We constructed additional molecules in which variable-region genes from S107 and DNS heavy chains (V_H S107 and V_H DNS) were joined to constant-region genes from kappa light chain (C_κ). Both of these chimeric molecules were expressed. Figure 5 shows the expression of V_H DNS-C_κ when it was introduced into a nonproducing myeloma. When V_H S107-C_κ was introduced into a kappa chain producer isolated from the S107 myeloma, it was not possible to demonstrate the reconstruction of an antigen binding site. This is in contrast to the previous results with the arsonate antibodies. The difference is probably due to the low affinity of the antibodies against phosphocholine. A single anti-phosphocholine site probably has such a low affinity for antigen that binding was not detectable with our assays. Similar results were obtained by Zack et al. (29); in mutants in which only H-L half-molecules were produced, the molecules could be retarded when passed through a phosphocholine-Sepharose column but they did not remain bound to the column. It is also possible to produce a chimeric light chain in which the variable region is of human origin and the kappa constant region is of mouse origin. When such a chimeric gene is transfected into mouse cells it is transcribed; the mRNA is translated into a protein which is the size of the light chain and remains intracytoplasmic (30).

Summary

Gene transfection is a powerful new tool for the study of immunoglobulin biosynthesis. It has proved useful in defining regulatory elements within immunoglobulin genes and should continue to be useful in the study of the developmental expression of immunoglobulin genes. Gene transfection is also a powerful technique for studying how the immunoglobulins function as proteins. It is possible with gene transfection to produce immunoglobulin molecules with novel chain combinations. This procedure has the advantage over reassembly in vitro that there is no denaturing step. Gene transfection has the additional advantage that it can be used to produce molecules with chimeric chains, which is not possible by assembly in vitro. Heavy chain variable region can be joined to kappa (light) chain constant region in a polypeptide; when the constant region is of mouse origin it has been possible to use variable regions of either mouse or human origin. It has also been possible to produce chimeric molecules with a heavy or light chain variable region of mouse origin and a heavy or light chain constant region of human origin. These molecules have the antigen-binding specificity of the mouse combined with the human effector functions. In the future it should be possible by gene transfection to produce molecules which are further modified by assembly of segments from different immunoglobulin chains and by mutagenesis in vitro.

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Effect of Somatic Mutation on Antibody Affinity and Specificity

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Abstract

By using antibody-forming cells in culture, it is possible to examine the effect of somatic mutations in the variable-region genes of antibodies in the absence of the selective forces that exist in vivo. Such studies reveal that single amino acid substitutions in the variable region of the heavy chain can lead to loss of antigen binding. An example is given of an important protective antibody against a bacterial antigen that undergoes a point mutation, resulting in loss of antigen binding and acquisition of autoreactivity.

The application of recombinant DNA technology to analysis of the structure and organization of immunoglobulin genes has revealed the major molecular events responsible for the generation of antibody diversity. Studies from many laboratories (1) clearly demonstrate that mature heavy chain and light chain genes are constructed from a number of distinct germline elements. Enormous sequence diversity is generated by the mixing and matching of these elements. Further diversity is created by the variation of recombination sites and the association of different variable regions of the heavy and light chains.

Once mature genes for the variable regions of heavy and light chains have been constructed and expressed, additional antibody diversity is generated by somatic mutation of these genes. Analysis of families of monoclonal antibodies, apparently derived from single germline heavy and light chain variable-region genes, reveals that nucleotide changes occur frequently in these genes and the sequences immediately flanking them. These changes accumulate during the course of B-cell differentiation (2–9). Although the exact mechanism and rate of somatic mutation have yet to be determined, it has been suggested that there is a mutation at every B-cell division (9), and most of the antibodies present in a secondary response differ from the germline by multiple amino acids (2–9).

While the studies cited above have provided great insight into the origins of the sequence diversity that is observed in antibodies, a number of crucial questions remain to be resolved. We do not know the exact mechanisms responsible for the nucleotide changes which occur in immunoglobulin genes that have already been rearranged and expressed. Most studies indicate that the changes are due to hypermutation (3). Some investigators suggested that an error-prone polymerase is responsible (10), while others suggested a mechanism similar to that of gene conversion (11, 12). Further, it is not clear whether

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somatic mutation is random, that is, affects all parts of the immunoglobulin gene equally. It is also not clear whether these changes are as likely to result in decreases as in increases in antigen binding. The studies of Kim et al. (3) and Gearhart and Bogenhagen (10) indicate that the variable region is the primary target of mutational changes. These workers observed that the changes were frequent in the coding and immediate 3′ and 5′ flanking sequences of the heavy and light chain variable region but were not found in more distant flanking sequences. The changes were also not found in the $C_H{}^1$ domain of the heavy chain and the constant region of the light chain. In fact, one could argue that the large intervening sequence between the J and C regions is there to separate the constant region from the variable region and thus protect it from somatic mutation.

Careful analysis of the accumulated base changes in families of monoclonal antibodies (2–10) derived from a single set of germline elements should help to answer these questions and also reveal the approximate rate at which somatic mutations occur. However, the fact that most of the monoclonal antibodies studied have affinities that are similar to or higher than those of the antibody encoded by the germline sequence (13, 14) indicates that the monoclonals analyzed are only a small subset of the products generated from that germline gene by somatic mutation. In fact, it is likely that antigen, T cells, and idiotype/anti-idiotype networks (15) have exerted significant selective pressure in vivo on the B cells that are amplified and available to be captured as hybridomas.

In an attempt to avoid this problem, we tried to answer a few very specific questions by using myeloma and hybridoma cells growing continuously in culture. We asked: (a) Do somatic mutants arise which have a diminished ability to bind antigen? (b) Will such mutants have single or multiple amino acid substitutions resulting from single or multiple base changes? (c) At what rate do such mutants arise? (d) Will antibodies with a decrease in binding for the original antigen acquire the ability to bind a different antigen?

We previously studied the S107 mouse myeloma cell line, which produces an immunoglobulin A (IgA) antibody that binds phosphorylcholine (PC). This antibody expresses the germline sequences of the T15 V1 and $J_H{}^1$ and of the $V_K{}^{22}$ and $J_L{}^5$ genes (16). It is also the predominant protective antibody made by BALB/c mice in response to pneumococcal and other bacterial polysaccharides (17). Furthermore, the three-dimensional structure of a closely related antibody has been determined (18).

We developed an immunoplate assay to isolate S107 subclones that are no longer able to bind antigen. Cells were cloned in soft agar and overlaid with antigen (PC-KLH) (Fig. 1). Clones which were not surrounded by a visible antigen-antibody precipitate were recovered from the agar, and the immunoglobulins produced by these clones were further characterized. A major problem with this approach is that even though antigen-binding mutants occur spontaneously at frequencies of 0.01 to 0.1%, mutants which are nonproducers and mutants which are low producers arise at similar frequencies. This means that every clone which is not surrounded by an antigen-antibody precipitate must be recovered and characterized in order to distinguish among these phenotypes. This makes it difficult to obtain large numbers of antigen-binding mutants.

At this time, only two mutants with decreased antigen binding and revertants from one of these have been fully characterized (19). The U1 mutant has a decreased ability to bind PC when it is attached to a carrier protein such as bovine serum albumin (PC-BSA) or in its natural state on bacterial polysaccharides (20). The covalent and noncovalent polymerization of U1 and the size of its heavy and light chains are indistinguishable from those of the parent. Chain recombination experiments revealed that the decrease in antigen binding is attributable to the U1 heavy chain. In collaboration with Stewart Rudikoff of the National Institutes of Health, we determined the amino acid sequence of the U1 heavy chain variable region and found a single amino acid substitution of an alanine for an aspartic acid at residue 101 (the fifth residue of J) (Fig. 3) (20).

Judging from the three-dimensional structure of McPC 603, an antibody closely related to S107, the amino acid substitution at residue 101 would be placed near the entrance to the binding site. Since chain recombination experiments revealed that the change in antigen binding was due to the heavy chain, we conclude that the substitution of alanine for aspartic acid in the fifth residue of J affects antigen binding.

Subsequent studies showed the U1 heavy chain variable region contains only a single base change (21). We therefore conclude that a single base change resulting in a single amino acid substitution can cause a significant decrease in an-

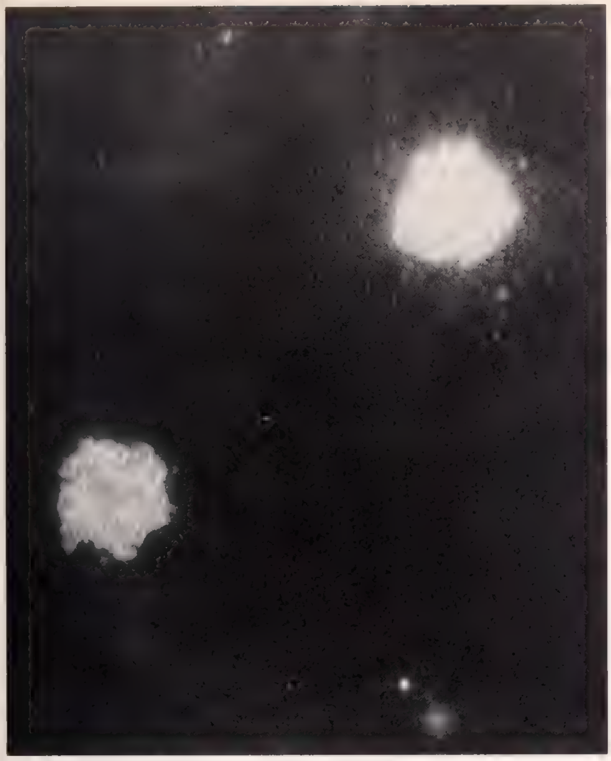

Fig. 1. Antigen overlay for detecting mutant cells. Cells of S107 line are cloned in soft agar and overlaid with antigen. Most cells are surrounded by antigen-antibody precipitate, which can be seen under low power with an inverted microscope (upper right). Rare mutant clones are not surrounded by a visible precipitate (lower left).

tigen binding and that mutants with point mutations in their variable-region genes arise spontaneously in myeloma cells in culture.

Similar conclusions can be drawn from studies of a second mutant, U4 (22). This mutant is even more interesting than U1 because it can be used to directly address some of the questions raised above. Equilibrium dialysis showed no detectable binding of phosphorylcholine by the U4 mutant (22). An enzyme-linked immunosorbent assay (ELISA) showed that U4 binds to PC-BSA poorly unless large amounts of PC are conjugated to BSA and large amounts of PC-BSA are adsorbed to the ELISA wells (Fig. 2). Even under those conditions, the binding of U4 was only a fraction of that of S107. Only when the incubation was allowed to continue for a long time (24 hr) and the binding of S107 exceeded the maximum detectable by the assay was the binding of U4 well above background. As can be seen in Fig. 3, the U4 heavy chain differs from the parental S107 germline sequence by a single amino acid substitution of an alanine for a glutamic acid at residue 35 of the first hypervariable region (22). Again, chain recombination experiments showed that the loss of antigen binding was due solely to the heavy chain. This dramatic loss of antigen binding associated with a single amino acid substitution is surprising, since the three-dimensional structure of McPC 603 indicates that at least four amino acid residues come in contact with phosphorylcholine (23).

The results described above can be explained by the fact that residue 35 plays a crucial role in the binding of this antigen. Analysis of the sequences of many heavy chain variable regions revealed that most PC-binding antibodies have a glutamic acid at residue 35, which is seldom seen in non-PC-binding antibodies (24). Second, three other germline genes are members of the S107 (T15) family (16); however, these closely related germline genes do not code for a glutamic acid at residue 35 (16) and do not encode PC-binding antibodies (2). Finally, on the basis of the three-dimensional structure of a closely related antibody, Davies and Metzger suggested that the glutamic acid at residue 35 is important in maintaining the integrity of the PC-binding site (23). Thus, the studies of the U4 mutant confirm that mutations arise frequently and spontaneously from the S107 cell line and that a single amino acid substitution can result in loss of antigen binding.

The U4 mutant can also be used to address the question of whether antibodies which have lost the ability to bind the original antigen acquire the ability to bind other antigens. Eilat et al (25) identified and sequenced a monoclonal autoantibody that bound DNA and was homologous to S107. Among other changes, it has a serine

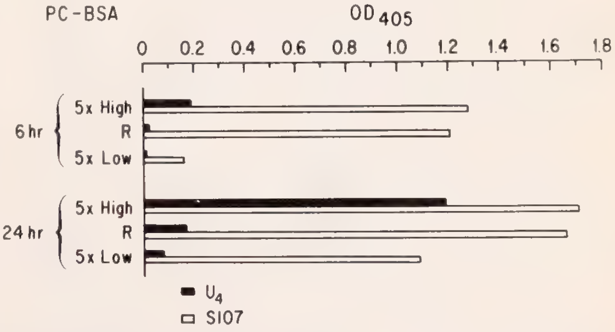

Fig. 2. Relative binding of U4 and S107 to phosphorylcholine-bovine serum albumin. BSA substituted with 3.05 moles of PC per mole of BSA (5 × high), 0.69 mole of PC per mole of BSA (R), and 0.80 mole of PC per mole of BSA (5 × low) were adsorbed to ELISA plates. The U4 or S107 immunoglobulin A antibody was incubated with the various antigen preparations. The wells were then washed and probed with alkaline-phosphatase-linked immunoglobulin A antibody for 6 or 24 hr. Absorbance at 405 nm (OD_{405}) was measured. It is important to note that the absorbance of the S107 on 5 × high at 24 hr exceeded the maximum sensitivity of our ELISA reader.

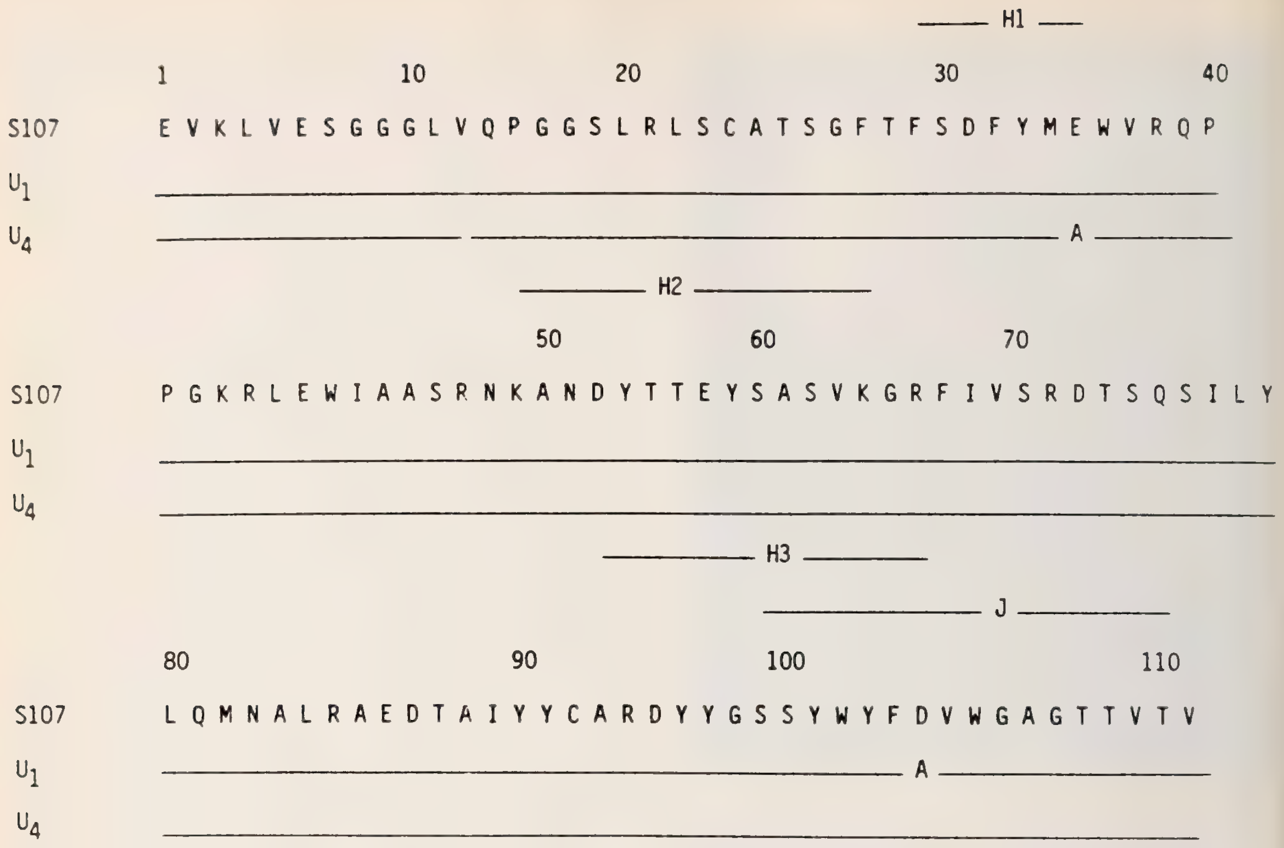
H1
1 10 20 30 40
S107 E V K L V E S G G G L V Q P G G S L R L S C A T S G F T F S D F Y M E W V R Q P
U1
U4 A
H2
50 60 70
S107 P G K R L E W I A A S R N K A N D Y T T E Y S A S V K G R F I V S R D T S Q S I L Y
U1
U4
H3
J
80 90 100 110
S107 L Q M N A L R A E D T A I Y Y C A R D Y Y G S S Y W Y F D V W G A G T T V T V
U1 A
U4

Fig. 3. Amino acid sequences of the U1 and U4 mutants. The sequence of the parental S107 and the mutant heavy chain variable regions is indicated by the one-letter code. The three hypervariable regions are denoted by H1, H2, and H3.

rather than a glutamic acid at residue 35. We therefore tested the reactivity of the parental S107 and the U4 and U1 mutants with a variety of autoantigens (26). As shown in the Table, U4 binds well to double-stranded DNA (dsDNA), the phospholipid cardiolipin, and phosphorylated protamine. This pattern of reactivity with phosphorylated antigens has been reported for autoantibodies from patients with lupus erythematosus and mice with a lupuslike syndrome (27, 28).

We therefore conclude that a single amino acid substitution not only can cause loss of binding to the eliciting antigen but also can result in acquisition of binding to another antigen. Even more interesting is the fact that a germline gene that encodes an important protective autoantibody can, as a result of a single somatic mutation, become the source of an autoantibody.

The mutation of the S107 germline gene which results in the formation of the autoantibody U4 may turn out to be a paradigm for the generation of a portion of the autoantibodies in autoimmune individuals. In light of this information, some of the questions that have been raised repeatedly about the origins of autoantibodies can be phrased more explicitly. For example, over the years a number of workers have suggested that microbial antigens may stimulate the production of antibodies which also react with self-components. Antibodies of the S107 (T15) family are elicited by various bacterial polysaccharides in mice, and in some strains, dominate the response to such antigens (17). If one or a few amino acid substitutions can convert one of these antibodies into an autoantibody, prior bacterial immunization is likely to lead to the appearance of autoantibodies through the somatic mutation of the T15 germline gene.

In fact, we examined MRL/1pr mice for antibodies against phosphorylcholine and against double-stranded DNA that express the T15 heavy chain variable-region gene(s). Only a few older autoimmune mice had high levels of such antibodies. We therefore attempted to increase the expression of the T15 idiotype in the mice by injecting newborns with immunoglobulin of the T15 idiotype (S107) and then immunizing 1 month later with phosphorylcholine attached to a protein carrier (KLH) (29). These mice and various controls began to develop autoimmunity and antibodies against double-stranded DNA. Al-

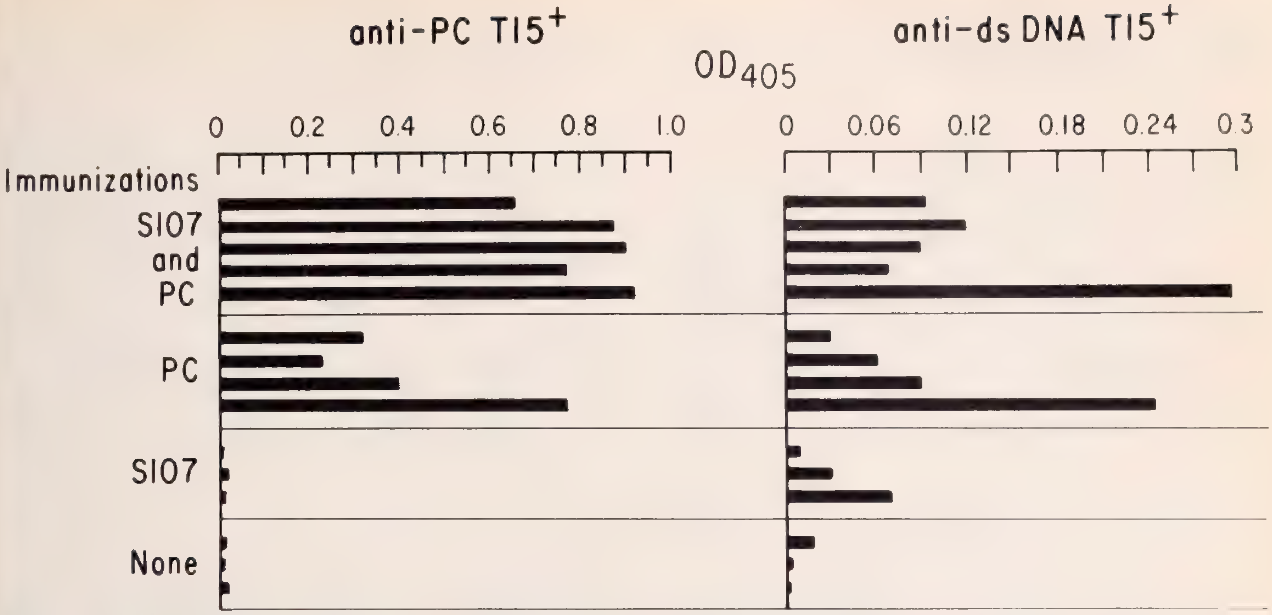

Fig. 4. MRL/1pr mice primed with S107 and immunized with phosphorylcholine were assayed for production of T15$^+$ antibodies against phosphorylcholine and double-stranded DNA. Phosphorylcholine-bovine serum albumin (10 μg/ml) or double-stranded DNA (5 μg/ml) was adsorbed to ELISA microtiter plates. After blocking with bovine serum albumin, dilutions of mouse serum (1:500) were added. Idiotype of bound antibody was determined with rat monoclonal antibodies against T15 which were probed with an alkaline-phosphatase-linked anti-rat antibody. Absorbance at 405 nm (OD_{405}) was measured. Background absorbance values of 0.043 and 0.281 for antibodies against phosphorylcholine and DNA, respectively, were subtracted. Background values were averages for five unimmunized MRL/1pr mice at 5 weeks of age. Each bar represents an individual mouse. The absorbances for the anti-PC and anti-dsDNA assays are not directly comparable because different amounts of antigen (phosphorylcholine or DNA) were bound to the plates.

though there was considerable variation from mouse to mouse and between litters, the data presented in Fig. 4 for such groups of mice at 8 weeks of age are representative of our preliminary studies. They show that mice which were primed neonatally with the T15 idiotype went on to make significant amounts of T15$^+$ PC-binding antibody. The PC$^+$T15$^+$ titers remained high over a period of months. Animals which were not primed neonatally but were immunized with PC-KLH also produced T15$^+$ PC-binding antibodies but did not sustain a high titer. Both groups synthesized easily detectable amounts of T15$^+$ antibodies against double-stranded DNA. Only occasional controls which were not exposed to either phosphorylcholine or T15 made detectable amounts of T15$^+$ antibody against double-stranded DNA. Monoclonal antibodies are now being analyzed to determine whether the genes which encode the antibodies against phosphorylcholine undergo somatic mutation to become genes coding for autoantibodies. These preliminary results suggest that the S107-U4 model may be realistic for autoimmune mice (26). Many more studies will be required to determine the importance of these antibodies in autoimmunity.

The studies described here only begin to address the questions of whether all or even most autoantibodies arise arise through somatic mutation, whether the rate of somatic mutation in autoimmune individuals is greater than that in normal individuals, and why such somatic mutants are produced in large amounts in autoimmune individuals and not in normal individuals. However, it should be possible to examine these questions by following the life history of certain

TABLE

*Binding of U4 and S107 Cells and Sera from MRL and BALB/c Mice to Phosphorylated Macromolecules**

	Binding (CPM) to			
Cells tested	PC-KLH	Double-stranded DNA (× 10)	Protamine	Cardiolipin
S107	4945	280	158	39
U4	781	1388	677	433
MRL serum 1/100	1425	1808	1545	2274
BALB/c serum 1/100	348	381	510	1043

* The macromolecules PC-KLH, phosphorylated protamine, and cardiolipin were adsorbed to polyvinyl microtiter wells and incubated with equal amounts of S107, U4, or diluted serum from unimmunized MRL/1pr or BALB/c mice. The amount of antibody bound to the wells was quantitated with ^{35}S-labeled monoclonal antibody against mouse kappa chain. The results are expressed as counts per minute (CPM) after subtracting backgrounds of 150 for PC-KLH, 122 (× 10) for double-stranded DNA, 246 for protamine, and 216 for cardiolipin. The binding of double-stranded DNA was determined by a filter binding assay.

variable regions in normal and autoimmune individuals and strains of mice.

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An Immunochemical and Molecular Analysis of Monoclonal Antibodies Bearing the A48 Regulatory Idiotype

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Abstract

Hybridomas that secrete antibodies bearing the ABPC48 regulatory idiotype were generated from BALB/c mice treated at birth or as adults with minute amounts of antibodies against A48 idiotype. The majority of these antibodies were recognized by IDA10, a syngeneic monoclonal antibody against A48 idiotype. In Northern blotting experiments, most of these hybridomas were shown to use heavy chain variable-region (V_H) genes related to the 441-4 germline V_H gene that encodes the heavy chain variable region of A48. Hybridization was detected between the V_H 441-4 probe and polyadenylated heavy chain mRNA isolated from the majority of the hybridomas. Southern blots confirmed these results by showing a rearrangement of V_H 441-4 related sequences to the heavy chain joining-segment clusters on the same hybridomas. The antibodies from all of the hybridomas derived from neonatal mice and from half of the hybridomas derived from adult mice showed specificity for fructosan determinants that, in most cases, was different from the specificity of A48 for the β-2,6-fructosan linkage. Surprisingly, several of the non-fructosan-binding hybridomas generated from the adult mice and the MOPC173 myeloma demonstrated a clear specificity for the β-1,6-D-galactan determinant. Of four galactan-binding myeloma proteins studied, XRPC44 alone shared idiotypy with the UPC10 myeloma. These findings suggest a possible mode of regulation in the immune system based on clonal cross-reactivity mediated by regulatory idiotopes.

Idiotypic interactions are the means by which the immune system can regulate the expression of its diverse resident lymphocytic clones. Numerous investigators have demonstrated that the appearance of auto-anti-idiotypic antibodies coincides with a decrease in the titer of idiotype-bearing antibodies during an immune response (1, 2). Furthermore, Rubinstein and Bona (3) showed that exposure to anti-idiotypic antibodies or the idiotype-bearing antibodies themselves, by placental crossing in utero or by parenteral administration to neonatal and adult mice, dramatically expanded the number of B-cell precursors bearing that idiotype. However, when working with an autologous system (i.e., the anti-idiotypic antibodies were elicited from the same mouse strain from which the idiotype-bearing antibodies were derived), we recently observed that idiotype-driven events were mediated by a special category of idiotopes, which we call regulatory idiotopes (4).

Regulatory Idiotopes

The concept of regulatory idiotopes emerged from an immunochemical analysis of four members of an idiotypic pathway initiated by A48, an immunoglobulin Ak, β-2,6-fructosan-binding myeloma protein which prototypically bears the A48 idiotype (A48-Id). Studying the idiotypy of polyclonal populations of syngeneic Ab_2 (anti-A48-Id), Ab_3 [anti-(anti-A48-Id)], and

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TABLE 1
Description of Monoclonal Antibodies Bearing the A48 Regulatory Idiotype

Antibody designation	Protocol for obtaining antibodies
A48, UPC10, MOPC173	from BALB/c myelomas
Series I 1-5-1(μ,k)	BALB/c mice injected at birth with 10 μg of A48 and challenged 1 month later with 20 μg of bacterial levan
Series II 2-1-3(γ3,k), 2-1-10(γ3,k), 2-11-1(μ,k), 2-28-9(γ3,k), 2-9-17(μ,k), 2-12-10(μ,k), 2-11-3(μ,k), 2-8-2(μ,k), 2-12-19(μ,k)	BALB/c mice injected at birth with 10 ng of syngeneic polyclonal antibodies against A48 idiotype and challenged 1 month later with 20 μg of bacterial levan
Series III 3-76-4(μ,k), 3-76-42(μ,k), 3-14-9(μ,k) 3-27-6(γ,k), 3-101-14(γ,k), 3-9-9(γ,k)	Adult BALB/c mice hyperimmunized with a conjugate of KLH and a syngeneic polyclonal antibody against A48 idiotype and challenged 1 month later with 20 μg of bacterial levan
Series AIDA AIDA 10-16(γ,k), AIDA 10-21(γ,k)	Adult BALB/c mice hyperimmunized with an IDA10-KLH conjugate
AIDA 23-3(γ,k), AIDA 23-3(γ,k)	Adult BALB/c mice hyperimmunized with an IDA23-KLH conjugate

Ab_4 {anti-[anti-(anti-A48-Id])} antibodies, we observed that the Ab_3 antibodies expressed the A48-idiotype since the Ab_2 and Ab_4 antibodies, respectively, bound to both the A48 and Ab_3 antibodies. Thus, we proposed that the particular idiotopes of Ab_1 (A48) which are immunogenic in a syngeneic system will elicit the production of Ab_2 antibodies. Parenteral administration of these antibodies will activate Ab_3 clones, which, for the most part, will express the A48 regulatory idiotype. Therefore, immunization with these Ab_3 antibodies will lead to the production of Ab_4 antibodies, which will be primarily antibodies against A48 regulatory idiotype. An idiotope is designated as a regulatory idiotope by demonstrating the following three properties: its ability to function as an autoimmunogen, its potential to become dominantly expressed due to recognition by regulatory T cells, and its expression by antibodies exhibiting different antigenic specificities (5).

By contrast, the population of antibodies which bear a given regulatory idiotype is not as precisely characterized. This leaves unanswered several basic questions about the nature of clones which bear and are subsequently regulated through these determinants. We have focused on two questions in particular. First, we know that anti-idiotypic antibodies very specifically activate clones bearing the complementary idiotype. However, the extent to which these activated idiotype-positive clones share antigen-binding specificity with the antibody used to generate the anti-idiotype is not clear. Second, it is not known whether the stably inherited regulatory idiotypes are germline-encoded sequences. Since regulatory idiotypes are almost always associated with heavy chain variable-region (V_H) determinants, they might represent phenotypic markers of V_H genes. This would imply that clones bearing a particular regulatory idiotype would use very similar or even identical V_H genes to encode the variable regions of their immunoglobulin heavy chains. Furthermore, it would represent an additional genetic criterion for defining the regulatory idiotope, which could now be distinguished from "conventional idiotypes" (à la Oudin) resulting from noniheritable somatic, recombinational, or mutational events.

Monoclonal Antibodies Bearing the A48 Regulatory Idiotype

To answer these questions, workers in our laboratory conducted a comparative analysis of the antigen-binding specificities and V_H gene origins of several monoclonal antibodies displaying the A48 regulatory idiotype and of A48 itself. Monoclonal antibodies bearing the A48 regulatory idiotype were prepared according to three independent protocols, listed in Table 1. These protocols have been described and have been shown to expand and activate the precursors of clones positive for the A48 idiotype (4, 6, 7).

Series I antibodies were derived from BALB/c mice injected at birth with the idiotype A48, series II antibodies from BALB/c mice injected at birth with syngeneic polyclonal antibodies against A48-Id, and series III antibodies from adult BALB/c mice hyperimmunized with a conjugate of KLH and a syngeneic polyclonal antibody against A48-Id. The series AIDA antibodies (kindly given to us by Dr. Pierre Legrain, Pasteur Institute, Paris) were generated in a similar

TABLE 2

Binding of the Monoclonal Antibodies to the IDA10 Reagent

Myeloma	Binding† (cpm)	Series I or II antibody	Binding† (cpm)	Series III or AIDA antibody	Binding† (cpm)
MOPC460‡	184 ± 17‡	1-5-1	991 ± 301	3-76-4	1548 ± 128
A48	5183 ± 289	2-1-3	679 ± 228	3-14-9	3745 ± 152
UPC10	1492 ± 141	2-8-2	804 ± 276	3-27-6	6030 ± 369
MOPC173	519 ± 233	2-11-1	728 ± 313	3-9-9	3078 ± 111
		2-12-10	1140 ± 136	3-101-14	2093 ± 41
		2-9-17	356 ± 257	AIDA 10-16	1616 ± 457
		2-28-9	795 ± 181	AIDA 10-21	3197 ± 674
				AIDA 23-2	9619 ± 107
				AIDA 23-3	9578 ± 553

* Microtiter plates were coated with monoclonal test antibodies at 5 μg/ml and then incubated with IDA10 antibodies against A48-Id. Following extensive washing, plates were then incubated with ^{125}I-labeled A48.

† Binding is expressed as average counts per minute (CPM) ± SE after subtraction of background (3% bovine serum albumin instead of monoclonal antibodies).

‡ MOPC460 is an immunoglobulin A_{κ} with DNP binding specificity.

way to the series III antibodies, except that the mice were immunized with KLH conjugates made with two different syngeneic monoclonal antibodies against A48-Id: IDA10 and IDA23. All hybridomas were initially screened for their ability to inhibit the binding of tritiated A48 protein to syngeneic polyclonal antibodies against A48-Id. Those which demonstrated this ability (Table 2) were retained for this study, and their expression of the A48 idiotype was further investigated in a radioimmunoassay with a panel of syngeneic monoclonal antibodies against A48-Id (kindly donated by Dr. Pierre Legrain) which all recognized antigen-inhibitable idiotopes on A48. Although the various monoclonal antibodies against A48-Id were bound by most of our monoclonal antibodies, only one, IDA10, was strongly bound by all of them. These results show that the IDA10 idiotope is a good candidate for an A48 regulatory idiotope.

Interestingly, when we used a constant protein concentration we observed differences in the extent of binding of IDA10 among these monoclonal antibodies. Based on the extent of this binding, the antibodies can be divided into two groups: series I and II antibodies, which bind IDA10 less well than A48, and series III and AIDA antibodies, which bind IDA10 as well as or even better than A48. These results suggest that the idiotopes being recognized by IDA10 may not be the determinant displayed by A48 and may reflect heterogeneity of heavy and/or light chain variable regions within this group.

The effect of heterogeneity in the light chain variable region (V_L) on A48 idiotype expression was evaluated by assaying the monoclonal antibodies for expression of a K light chain belonging to the V_K10 family. From protein sequence data it is known that UPC10, another β-2,6-fructosan-binding myeloma which shares idiotypy and V_H gene origin with A48 (8, 9), has a V_K10 light chain (10).

Monoclonal antibodies positive for the A48 idiotype were labeled with alkaline phosphatase, and their direct binding to rabbit antibodies against V_K10 was measured with an enzyme-linked immunosorbent assay (ELISA). No correlation was evident between expression of the V_K10 light chain and strength of binding to the IDA10 reagent. These results support the idea that the A48 regulatory idiotype is associated with V_H determinants, but they do not exclude the possibility that three-dimensional structures associated with the various V_K families sterically interfere with the binding of IDA10 antibodies, although we consider such steric interference unlikely.

The V_H gene origin of these monoclonal antibodies was more extensively analyzed by Northern blotting techniques (15). Both A48 and UPC10 derive their rearranged and expressed V_H gene from the V_H 441-4 germline gene (9). This gene and the V_H X24 germline gene are the only members of the V_H 24 germline gene family, which lies at the 5′ end of the cluster containing all the germline V_H families on chromosome 12 (11). A V_H 441-4 germline gene probe (the V_H Ga139 probe, kindly given to us by Dr. S. Rudikoff, National Cancer Institute, Bethesda, Maryland) was nick-translated with phosphorus-32-labeled deoxynucleotide triphosphates and hybridized to Northern blotted polyadenylated cytoplasmic RNA from each hybridoma from series I, II, III, and AIDA. With a majority of these A48-idiotype-positive hybridomas, we observed hybridization of this probe to a messenger RNA (mRNA) species whose size is consistent with the isotype of the antibody produced by the hybridoma. When the RNA from all the hybridomas to which the probe did not hybridize (i.e., series II antibodies 2-8-2, 2-11-3, 2-9-17, and 2-12-9 and series III antibodies 3-76-4 and 3-76-42) was hybridized to the appropriate heavy chain isotype probe, only 3-76-4 and 3-76-42 failed to hybridize. These two hybridomas are currently being recloned.

In all the hybridomas for which we observed hybridization of heavy chain mRNA to the V_H 441-4 probe by Southern blotting analysis, we observed a rearrangement to the JH cluster of a V_H gene belonging to the X24 V_H family. Genomic DNA was digested with *Eco* RI and Southern blotted (15). Duplicate blots were hybridized with the V_H 441-4 probe as well as a JH_3-JH_4 probe to confirm the identity of the rearranged band. Interestingly, all the hybridomas from series I and II showed hybridization of the V_H 441-4 probe to the same rearranged *Eco* RI fragment at 2.91 kilobases (kb), which is different from the A48 type of rearrangement. Among the hybridomas from series III and the AIDA series, we saw five different sizes of rearranged bands (2.95, 2.55, 2.4, 2.2, and 1.65 kb), including the one observed in A48 (2.2 kb). These sizes are consistent with the rearrangement of V_H 441-4 or V_H X24 germline gene to each of the four different JH sequences.

Taken collectively, the data obtained by Northern blotting and Southern blotting indicate that most of the hybridomas from series II and all of the hybridomas from series III and AIDA utilize a V_H gene to build their A48-idiotype-positive antibody which is highly homologous to the V_H 441-4 germline gene expressed by A48 itself. The technique of primer extension is being used to sequence the heavy chain mRNA from several representative hybridomas from each series. This sequence information will be vital not only for understanding the basis for the heterogeneity of A48 idiotype expression among these hybridomas but also ultimately for determining the molecular basis for expression of the A48 regulatory idiotype.

Antigen-Binding Specificities of Monoclonal Antibodies

Using a radioimmunoassay, we investigated the antigen-binding specificities of these monoclonal antibodies by testing their ability to bind grass levan (100% β-2,6-fructosan linkages), bacterial levan (β-2,6-fructosan linkages with β-2,1-fructosan linkages at the branch points), and inulin (100% β-2,1-fructosan linkages). As previously reported, A48 and UPC10 are specific for the β-2,6-fructosan linkage. Hence, they bind to both grass and bacterial levan but not to inulin. On the other hand, J606, which is specific for the β-2,1-fructosan linkage, binds to inulin and bacterial levan but not to grass levan (12). Therefore, by studying the pattern of binding of these antibodies to each of these polyfructosans, their precise linkage specificity, if any, can be determined. The results of this experiment are summarized in Table 3. All ten of the antibodies in series I and II bound to bacterial levan quite well. However, only two of them (2-8-2 and 2-11-1) showed specificity for the β-2,6-fructosan linkage by binding to rye levan as well. Five antibodies (1-5-1, 2-1-10, 2-11-3, 2-12-10, and 2-28-9) bound only to bacterial levan, which indicates that these antibodies are specific for a conformational determinant present only in bacterial levan. Two antibodies (2-1-3 and 2-12-19) bound to all three polysaccharides. However, the binding of these two antibodies to bacterial levan was not inhibited by either inulin or grass levan. These results suggest that although the combining site of these antibodies could accommodate either the isolated β-2,6 or the β-2,1-fructosan linkage, they recognize with a much higher affinity a conformational determinant present only in the bacterial levan polysaccharide. One antibody (2-9-17) clearly showed specificity for the β-2,1 linkage by binding to both bacterial levan and inulin.

In series III and the AIDA series, only two of ten antibodies (3-14-9 and AIDA 10-16) showed any affinity for bacterial levan. Antibody 3-14-9 bound to all three polysaccharides, and AIDA 10-16 bound only to bacterial levan; thus, they are probably both specific for a conformational determinant in the bacterial levan polysaccharide, as explained above. Only three other antibodies from series III showed any specificity for a fructosan determinant; 3-76-42 bound to grass levan and inulin, 3-27-6 to grass levan, and 3-101-4 to inulin. Recent sequencing data showed that the β-1,6-D-galactan-binding myeloma proteins derive their rearranged V_H genes from the V_H 441-4 as well as the V_H X24 germline genes (13). In fact, these two genes differ only by seven nucleotide substitutions (14). This observation prompted us to assay for β-1,6-D-galactan binding activity the series III and AIDA antibodies which exhibit the A48 idiotype but no specificity for any fructosan determinants. A radioimmunoassay was used to compare the

TABLE 3
Antigen-Binding Specificity of Monoclonal Antibodies

	Binding (CPM) to antigen*					
Antibody designation	Rye levan (20 μg/ml)	Bacterial levan (20 μg/ml)	Inulin (5 μg/ml)	Arabinogalactan (20 μg/ml)	Galactan-BSA† (5 μg/ml)	Gum ghatti (20 μg/ml)
MOPC460	225 ± 79*	376 ± 116	44 ± 2	—	—	—
A48	5,659 ± 275	12,819 ± 945	91 ± 5	299 ± 199	357 ± 105	191 ± 07
UPC10	1,353 ± 95	19,233 ± 723	188 ± 30	—	—	—
MOPC173	194 ± 32	234 ± 45	67 ± 21	—	—	—
J606	360 ± 87	4,265 ± 833	5,875 ± 419	—	—	—
XRPC24	—	—	—	872 ± 13	5,272 ± 161	3,537 ± 51
1-5-1	819 ± 272	12,576 ± 1,867	223 ± 53	—	—	—
2-1-3	4,013 ± 328	12,570 ± 903	8,347 ± 1,024	—	—	—
2-1-10	802 ± 222	8,503 ± 1,516	249 ± 147	—	—	—
2-8-2	1,057 ± 161	16,232 ± 1,418	98 ± 47	—	—	—
2-11-1	1,592 ± 206	13,286 ± 805	186 ± 40	—	—	—
2-11-3	102 ± 3	12,354 ± 697	151 ± 26	—	—	—
2-12-10	113 ± 76	11,230 ± 1,650	40 ± 13	—	—	—
2-12-19	2,528 ± 408	15,028 ± 1,095	1,453 ± 527	—	—	—
2-9-17	62 ± 15	13,434 ± 2,915	2,228 ± 645	—	—	—
2-28-9	666 ± 294	5,232 ± 1,421	61 ± 21	—	—	—
3-76-4	550 ± 69	127 ± 1	77 ± 12	—	—	—
3-76-42	1,544 ± 352	190 ± 41	1,171 ± 271	—	—	—
3-14-9	5,623 ± 947	1,413 ± 263	3,583 ± 733	—	—	—
3-27-6	1,693 ± 293	96 ± 11	177 ± 88	—	—	—
3-9-9	118 ± 30	126 ± 14	105 ± 19	273 ± 45	183 ± 21	189 ± 15
3-101-14	103 ± 19	152 ± 12	1,482 ± 275	—	—	—
AIDA 10-16	179 ± 119	2,888 ± 291	125 ± 89	—	—	—
AIDA 10-21	143 ± 76	294 ± 145	486 ± 235	730 ± 352	575 ± 64	465 ± 107
AIDA 23-2	311 ± 86	151 ± 125	149 ± 34	1,709 ± 324	1,832 ± 300	1,376 ± 54
AIDA 23-3	164 ± 44	405 ± 127	851 ± 180	296 ± 112	709 ± 110	483 ± 96

* Binding is expressed as average counts per minute (CPM) ± SE for triplicate determinations.
† BSA, bovine serum albumin.

binding of these A48-idiotype-positive antibodies, A48 itself, and XRPC 24 (a β-1,6-D-galactan-binding myeloma protein) to the β-1,6-D-galactan determinants in galactan-BSA and in two naturally occurring heteropolysaccharides, arabinogalactan and gum ghatti (Table 3). One AIDA antibody (AIDA 23-2) clearly showed a specificity for β-1,6-D-galactan determinants. Two others (AIDA 10-21 and AIDA 23-3) exhibited galactan-binding activities that were much lower but nevertheless well above negative control values. This intriguing demonstration of A48 idiotype expression by galactan-binding antibodies was supported by a further observation. We studied four galactan-binding myeloma proteins (XRPC24, XRPC44, TEPC601, and J539) and found that XRPC44 could competitively inhibit the binding to UPC10 of syngeneic polyclonal antibodies against UPC10. As previously mentioned, UPC10 extensively shares idiotypy with A48 as well as the β-2,6-fructosan antigen specificity and V_H 441-4 germline gene origin of its expressed V_H.

Thus, only one series III antibody (3-9-9) of the 20 antibodies analyzed in this study has turned out to be a true Ab_3 by the criterion that it does not bind to any fructosan or galactan determinants.

Function of Regulatory Idiotypes in the Immune System

Collectively, the data presented in this paper illustrate several key points concerning the function of regulatory idiotypes in the immune system. Before discussing these points, however, it is important to emphasize the syngeneic origin of all the anti-idiotypic antibodies used in these experiments. By definition, only antibodies to idiotopes which are autoimmunogenic will be present. Hence, the action of these anti-idiotypic antibodies on the immune system provides an appropriate model for describing immunoregulation through the A48 regulatory idiotype.

The first point illustrated by these experiments is the relative ease with which minute amounts of idiotype-bearing or anti-idiotypic antibody can upset the idiotypic equilibrium which is maintained among the clones populating the immune system. Through the action of anti-idiotypic antibodies specific for the A48 regulatory idiotype, B-cell precursors bearing the A48 idiotype, a normally silent component of the bacterial levan immune repertoire, are expanded and dominantly expressed at the expense of the other clones, which are now functionally suppressed.

By immortalizing as hybridomas the A48-idiotype-positive clones, derived from animals in which the A48-idiotype-positive precursors were expanded by anti-idiotypic antibodies, we have a unique opportunity to characterize a population of clones that are all products of immunoregulation through a shared regulatory idiotype. These A48-idiotype-positive hybridomas were compared to A48 in three different areas: idiotype expression, V_H gene origin, and antigen-binding specificity. In the first two areas in particular, we noted many similarities to A48 but also some important differences. As anticipated, there are A48 idiotype determinants which are shared by all the monoclonal antibodies. One monoclonal antibody to the A48 idiotype (IDA10) reacts with all the A48-idiotype-positive (A48-Id$^+$) hybridomas. However, there are quantitative differences between the A48-Id$^+$ monoclonal antibodies in this reaction with IDA10, and this is our first indication of the diversity which apparently exists in this population.

By Northern blotting analysis, we demonstrated that the vast majority of these A48 RI bearing hybridomas derive their expressed V_H gene from the same X-24 V_H germline gene family from which A48 derives its V_H gene. This strongly supports the concept of regulatory idiotypes being V_H germline gene markers. However, it does not imply that there is 100% nucleotide sequence homology between the V_H genes expressed by the various hybridomas. In fact, we would expect the structural correlate(s) required to form the A48 regulatory idiotype to account for a very small proportion of the V_H gene sequence. Such V_H sequence heterogeneity, and possibly V_L sequence heterogeneity as well, could account for the heterogeneity in A48 idiotype expression which was previously noted. This issue will be directly addressed in the experiments being carried out to sequence the heavy and light chain variable regions of these A48-Id$^+$ monoclonal antibodies.

The antigen-binding analyses of these hybridomas yielded the most unexpected but perhaps most meaningful results of these studies. As anticipated, most of these antibodies did bind BL and were therefore not of the Ab_3 type, but only a very minor proportion displayed the same specificity for the β-2,6-fructosan linkage as A48. Most intriguing was the discovery that several of the A48-Id$^+$ clones which were not specific for any fructosan determinant bound quite well to β-1,6-D-galactan determinants. This observation pointed out one of the most fundamental aspects of network regulation through regulatory idiotopes, which we have designated as "cross-regulation." We define the cross-regulatory process to include those humoral and cellular mechanisms which, through the self-recognition of regulatory idiotypes, control discrete populations of clones that may express diverse antigen-binding specificities.

Indeed, our data make precisely this point. Antibodies against the A48 regulatory idiotype can activate a population of B-cell clones which exhibit enough genetic homology to express sufficient levels of the A48 regulatory idiotype that they are all cross-regulated. Nevertheless, they are heterogeneous enough to show diverse antigen-binding specificities.

Thus, the types of clones which are expanded as a result of cross-regulatory processes will differ substantially from those expanded subsequent to an immune response to a foreign antigen. Stimulation by an antigen activates only clones which are specific for that antigen, but the clones may bear diverse idiotypes depending on the number of distinct epitopes presented by that antigen. On the other hand, antibodies against regulatory idiotypes specifically activate only clones bearing the complementary idiotype, but the antigen specificities of these clones may be considerably more diverse. Their range of antigen-binding specificities would, however, be limited to the types of antibody combining sites which could be formed by the V_H genes belonging to the one V_H family for which this particular regulatory idiotype is a phenotypic marker.

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Structure and Function of Murine Class II Major Histocompatibility Complex Genes

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The I region of the murine major histocompatibility complex (MHC) was first identified as a locus containing genes controlling specific immune responses (Ir genes) (1). The same chromosomal segment was also found to include genes encoding highly polymorphic membrane antigens termed Ia (for *I*-region-*a*ssociated) antigens (2). A combination of immunogenetic, serologic, and biochemical investigations demonstrated that the Ia antigens are heterodimeric cell surface glycoproteins, consisting of one heavy (α) and one light (β) chain in noncovalent association (3). Two isotypic forms of Ia are found in mice: I-A (A_β:A_α) and I-E (E_β:E_α). We now know that Ir genes are the structural genes for Ia molecules, and that the involvement of these Ia molecules in T-lymphocyte development and activation is responsible for Ir gene phenomena (4).

Recently, the genes encoding the α and β chains of Ia were cloned and analyzed (3). This has permitted a detailed understanding of the complete protein sequence of allelic forms of each Ia subunit. DNA-mediated gene transfer has begun to be employed in correlating protein structure with function. We summarize here our own work and that of others on the organization of class II genes and the relationship of allelic and isotypic variation to expression and immunologic activity.

Organization of the I Region

By utilizing available recombinant DNA clones of either human or mouse class II genes corresponding to the well-characterized Ia protein products, two laboratories have combined cosmid cloning with chromosomal "walking" techniques to generate a molecular map of the I region of the mouse (5, 6). As shown in Fig. 1, the four known class II genes are found in a 100-kilobase (kb) region of DNA in the order: centromere–Aβ–Aα–Eβ–Eα. In addition to these expected genes, four other homologous regions of DNA have been identified: $A\beta_3$, about 70 kb 3′ of the K-end cluster of class I genes; $A\beta_2$, about 20 kb 5′ of Aβ; $E\beta_2$, between Eβ and Eα; and $E\beta_3$, an undetermined distance 3′ of Eα. The overall structure and possible significance of these new class II "genes" are under investigation (see below).

Some unexpected findings emerged from this work. First, almost all recombination within the I region seems to occur in a small (approximately 2 kb) stretch of DNA within an intron of the Eβ gene (5, 7). Second, no obvious complete class II genes map to the regions defined as I-B and I-J by functional studies, and thus the relationship between I-region gene products and the structures involved in immune phenomena associated with the I-B and I-J regions is not clear (5, 7, 8).

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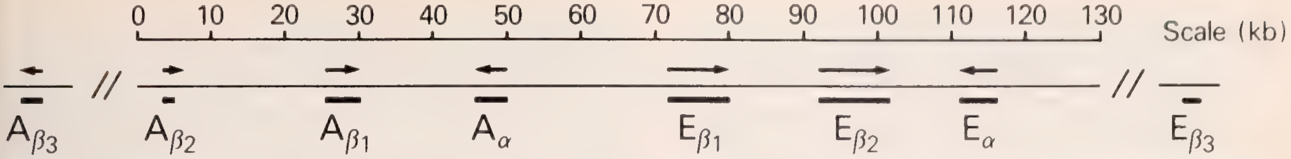

Fig. 1. Molecular map of murine I region. Map is oriented with chromosome 17 centromere to left. Thick black lines above gene symbols ($A\beta_1$, $A\alpha$, etc.) indicate known span of gene. Additional regions of genes such as $A\beta_2$, $A\beta_3$, and $E\beta_3$, which have not been fully mapped, are not included. Arrows show direction of transcription.

Structure of Class II Genes

Efforts in several laboratories have led to the elucidation of the complete genomic organization of three of the four major class II genes (Aβ, Eβ, and Eα) [reviewed in (3, 9)] (see Fig. 2). (Although Aα has been cloned, its organization has not been published.) The Aβ and Eβ genes each consist of six exons, the first of which encodes the signal peptide and the first several amino acids of the mature NH_2 terminus. The second through fourth exons encode the remainder of the NH_2-terminal domain (β_1), the membrane-proximal domain (β_2), and the hydrophobic transmembrane (TM) region, respectively. The fourth exon also contains some codons for intracytoplasmic (IC) amino acids. The intracytoplasmic region is completed by a fifth exon and the very beginning of the sixth exon, which also contains the 3′ untranslated (3′ UT) region and polyadenylation signal. The Eα gene differs from the β genes only in having the transmembrane and intracytoplasmic regions and the first part of the 3′ untranslated region on a single exon, separated from the remainder of the 3′ untranslated region and polyadenylation signal. In the 5′ flanking DNA, a pair of highly conserved sequences has been identified in all class II genes studied to date (10, 11). The function of these sequences is not known, but is presumed to be related to regulation of the expression of class II genes.

We have also analyzed the $E\beta_2$ gene. Contrary to the original report of Steinmetz et al. (5), which only demonstrated a sequence homologous to the 3′ half of the DQβ molecule, we found that $E\beta_2$ contains apparently normal signal peptide,

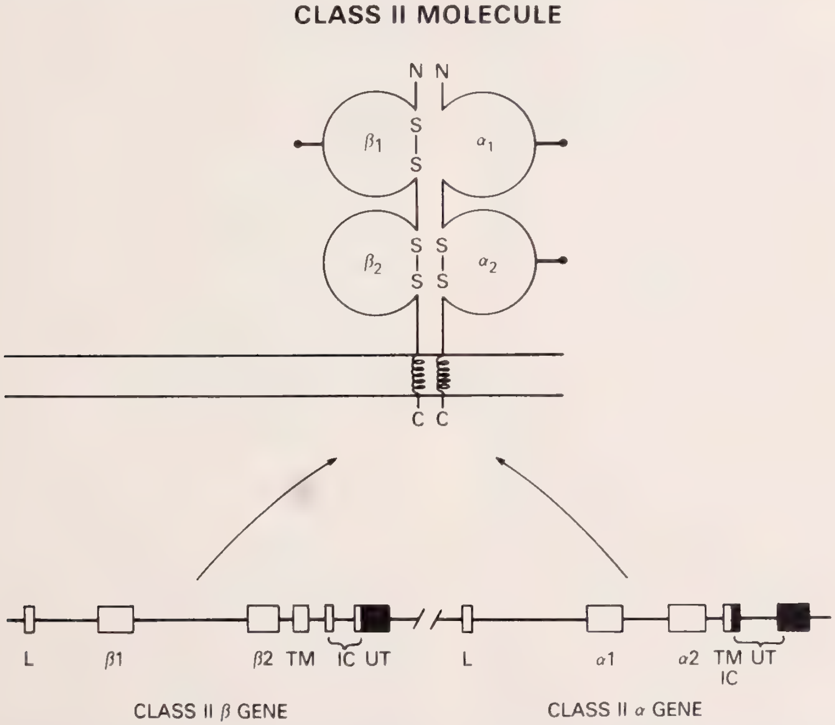

Fig. 2. Intron-exon organization of murine class II genes. Boxes represent exons, approximately to scale. Open boxes represent coding regions, filled boxes untranslated regions. Thin lines represent intervening sequence and flanking DNA. L, exon encoding leader (signal peptide); β_1 and α_1, exons encoding NH_2-terminal domains; β_2 and α_2, exons encoding membrane-proximal domains; TM, exon encoding transmembrane segment; IC, exon(s) encoding intracytoplasmic region; UT, exon(s) encoding 3′ untranslated region.

β_1, and β_2 exons. A transmembrane region is present, but the organization of the intracytoplasmic and 3′ untranslated regions is not yet clear (NSB and RNG, unpublished observations). The conserved 5′ flanking nucleotides are present in the $E\beta_2$ gene, and low levels of messenger RNA (mRNA) transcripts have been found in spleen cells and B-cell tumors or hybridomas as well as L-cell transfectants. The possible protein product and functional role of $E\beta_2$ are under active study.

Several allelic forms of each major class II gene have been sequenced (12–19), providing insight into the sites of intraspecies polymorphism believed critical to the immune function of Ia molecules. These studies revealed a predominance of amino acid variation in the NH_2-terminal (α_1 and β_1) domains of the $A\alpha$, $A\beta$, and $E\beta$ molecules. ($E\alpha$ appears to be significantly less polymorphic.) Within these NH_2-terminal domains, certain regions show greater variability than others, allowing the definition of three or four hypervariable (HV) regions, which occur around positions 1–10, 25–35, 65–75, and 85–95.

Ia Expression Following Transfer of Class II Genes

To analyze in detail how the structure of Ia molecules is related to their function, use has been made of the expression of cloned genes in cultured cells following DNA-mediated gene transfer. Initial experiments focused on the use of B-lymphoma cells as recipients, since these Ia-expressing tumors would be expected to efficiently transcribe and translate the introduced class II gene, and could assemble the product of a single transferred class II gene with an endogenous complementary chain. Workers at our laboratory (20) and that of Seidman (21) showed that Ia^+ Balb/c (Ia^d) lymphoma cells expressed a transfected A^k_β gene, apparently as an $A^k_\beta{:}A^d_\alpha$ dimer. The expressed A^k_β was serologically similar if not identical to that expressed by F_1 (k × d) B-cell hydridomas, and its expression by an Ia^d-positive cell led to the appearance of new, T-cell-recognized allodeterminants.

To gain better control over the precise class II gene products expressed by a cell, we and others turned to Ia^- recipient cells, in particular mouse L-cell fibroblasts (22–25). It was easily shown that although no single class II gene was expressed by itself after transfer to L cells, the products of pairs of α and β genes from the same haplotype were readily expressed at the cell surface. Further, by appropriate use of strong promoter/enhancer elements or cell sorting, transfectants with levels of Ia comparable to those of B-cell tumors could be isolated. However, during attempts to create L cells with haplotype-mixed molecules such as $A^k_\beta{:}A^d_\alpha$, an unexpected observation about Ia assembly and/or expression was made (34).

In contrast to the described results on B-lymphoma cells, cotransformation of L cells with A^k_β and A^d_α did not lead to detectable expression of Ia at the cell surface. Since such molecules could be seen on the transfected B cells, the monoclonal antibodies being used were apparently not missing them. Comparison of the mRNA levels in B-lymphoma cells with those in the transfected L cells suggested that the problem in the L cells might be quantitative. The L cells were therefore transfected with A^k_β and a new A^d_α gene construct which gave increased expression. The result was cells with a low but detectable level of expression of Ia protein at the surface. Repetitive cell sorting permitted isolation of cells with a surface Ia level comparable to those of $A^k_\beta{:}A^k_\alpha$ transfectants. Northern blots for $A\beta$ and $A\alpha$ mRNA in such pairs of cells showed that to achieve the same level of surface expression, substantially greater amounts of Ia mRNA were necessary in the mixed than in the haplotype-matched situation. Thus, it appears that certain $\alpha{:}\beta$ pairings are quantitatively less efficient at assembly and/or expression. The defect in surface expression can be overcome by increasing the available α and β chains in the cell. Although this has been studied in full detail only for d:k pairs, recent work with permuted pairs involving $A^{b,d,k}_\beta$ and $A^{b,d,k}_\alpha$ indicates a hierarchy of assembly potential. Pairs derived from the same haplotype are always as or more efficient than mixed pairs. Some combinations such as $A^b_\beta{:}A^k_\alpha$ are almost as well expressed as the haplotype-matched pairs, while others (e.g., $A^d_\beta{:}A^k_\alpha$) are extremely hard to generate.

The segment of the β chain controlling the assembly/expression just described has been partially mapped. It is possible to interchange portions of allelic genes by an "exon-shuffling" procedure utilizing genomic clones (Fig. 3). Using this general approach, it has been found, as might be predicted, that the highly polymorphic β_1 domain is critical to the variation in expression seen with different $A\alpha$ chains. Experiments are in progress to dissect out the subregions within the β_1 domain which are important in this process and to analyze the $A\alpha$ gene in a similar manner.

Along with these experiments on intraisotypic mixing, we have begun to look at the regulation of interisotypic pairing, that is, Aβ with Eα, Eβ with Aα. The initial control experiment was designed to demonstrate the expected failure of Aβ to pair with Eα, and hence provide the test situation for shuffling Aβ with Eβ and Aα with Eα to map the regions involved in isotype-selective association. Surprisingly, this experiment showed assembly and expression of an A_β^d:E_α^k dimer by transfected L cells. This unexpected result was confirmed with several gene constructs in independent experiments, and immunoprecipitation studies with two-dimensional gel analysis indicate that the A_β^d and E_α^k polypeptides are physically associated in an Ia-type heterodimer (35). Interestingly, it has not been possible to achieve expression of A_β^k with E_α^k, suggesting that, at a minimum, the β_1 domain polymorphism critical for Aβ-Aα pairing may also be important in mixed-isotype pairing. This would be in addition to any as yet undetected control of dimer formation by the A or the E isotype. Experiments with hybrid α and β gene constructs of various allelic origins are under way to further explore this intriguing problem.

Functional Studies of Transfected Class II Genes

The class II gene transfectants described above have been used to explore the relationship between Ia structure and serologic or T-cell-mediated recognition of these molecules. To date, all transfected cells, either B-lymphoma cells or L cells, show the same serologic profiles with a large panel of monoclonal antibodies against Ia antigens as do control cells expressing endogenous Ia genes (23–26). Immunoprecipitation studies indicate that the two-dimensional electrophoretic patterns of the Ia molecules expressed by transfected B cells and L cells are similar or identical to those of endogenous Ia products, indicating normal posttranscriptional and posttranslational processing.

To investigate which portions of Ia molecules are critical to allele-specific recognition of Ia by antibodies, various recombinant Ia products have been analyzed. Expression of an exon-shuffled Aβ gene containing L^k and β_1^k, $[\beta_2$, TM, IC, 3′ UT$]^d$ cotransfected with A_α^k, generates a molecule with a serologic profile identical to that of wild-type A_β^k:A_α^k pairs. Thus, the highly polymorphic $A\beta_1$ domain is essential for the creation of A_β^k serologic specificities. However, this experiment did not address the question of whether these antibodies directly "see" or bind to the $A_{\beta 1}^k$ domain exclusively, or whether the β_1 polymorphic structure influences the configuration of the entire I-A^k molecule to stabilize or even generate the relevant epitopes. To look at this issue, we created a hybrid class II- class I molecule containing the L and β_1 exons of A_β^k and the α_3[C2], transmembrane, intracytoplasmic, and 3′ untranslated portions of the class I D^d gene (27). Such a chimeric gene is expressed by transfected L cells. Staining with monoclonal antibodies against I-A^k, which were previously shown by analysis of the A_β^k:A_α^d-expressing B lymphoma transfectant and the $\beta_1^k[\beta_2$, TM, IC$]^d$ exon-shuffled gene transfectant to require the $A_{\beta 1}^k$ domain for recognition, gave variable fluorescence with cells expressing this new gene product. The antibody 10.2.16 interacted strongly with this hybrid product, whereas antibodies 40F, 40L, and 40M showed little reactivity and 39B, 39E, and 40A showed no reactivity. Thus, while the serologic epitopes seen by all these antibodies *require* the $A_{\beta 1}^k$ domain, this domain does not constitute the sole component of such epitopes in most cases. These results suggest that caution should be used in assigning reactivities to regions or amino acids on the basis of homologous gene constructs alone. The sites which are shown by such experiments to be essential or necessary to the epitope being analyzed may not be sufficient for its creation.

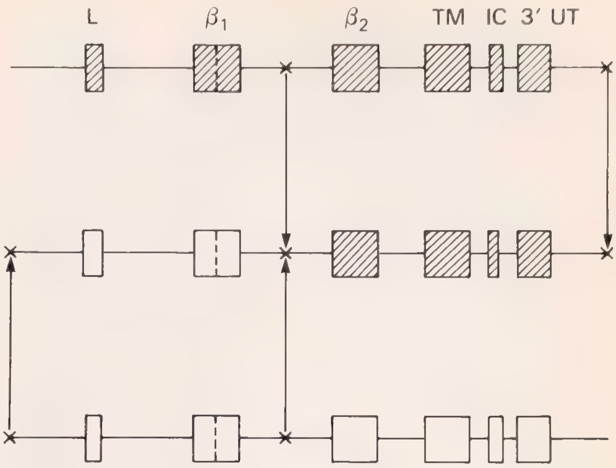

Fig. 3. General strategy for class II β gene exon shuffling. Two allelic forms of Aβ gene are shown at top (hatched boxes) and at bottom (open boxes), respectively. Fragments of genomic DNA clones used in construction of shuffled gene (middle) are indicated by X's and arrows. Symbols are defined in legend of Fig. 2.

The main purpose of the transfection experiments has been to determine how the structure of Ia is related to Ia-restricted recognition of antigen by T cells. The B-lymphoma cells known to function as accessory cells for T-cell stimulation have been shown to present antigen in studies of

Ia products of transfected genes (20). However, much of the interesting data on this topic has been derived from experiments with transfected L cells. Two groups have found that L cells which express Ia are capable of acting as antigen-presenting cells (APC) to T-cell hybridomas (24, 25), and our studies have extended this to the stimulation of long-term T-cell clones and primary allogeneic or mitogen-induced proliferative responses of virgin T cells (25). The findings indicate that L cells have whatever is required besides Ia to serve as accessory cells in a variety of T-cell activation systems. This provides an excellent model for dissection of Ia structure and function, since the *only* class II molecules expressed are those introduced experimentally.

With rare exceptions, L-cell transfectants expressing Ia molecules have been able to stimulate all T cells specific for the antigen plus the allelic form of Ia on the L-cell membrane. In several cases in which the L-cell transfectants were unable to stimulate the T cells, the inactivity could be overcome by increasing the number of transfectants or the antigen concentration used in the assay. Careful dose-response experiments with L cells transfected with the same genes, but sorted to bear varying levels of surface Ia, showed a clear relation between Ia density and antigen dose required for T-cell activation, similar to the [Ia × ag] product relationship reported by Matis et al. (28) for conventional antigen-presenting cells.

Antigen processing by accessory cells plays an important role in Ia-restricted presentation of complex antigens to T cells (29, 30). Since L-cell transfectants that express Ia stimulated T cells known to require processed antigen for recognition, it seemed likely that L cells possessed the necessary "processing" machinery. This was formally demonstrated by experiments with aldehyde fixation or lysosomatropic agents previously employed with splenic antigen-presenting cells or B-cell tumors. Transfectants exposed to antigen and then treated to prevent further processing could stimulate T-cell hybridomas, but transfectants treated before antigen exposure could not (36). These findings imply that the metabolic mechanisms for processing antigen may be widely distributed among different cell types and may not be confined to specialized antigen-presenting cells.

Two other aspects of the general interaction between T cells and antigen-presenting cells have been studied in this model. First, the ability of antibody against L3T4 to block the stimulation of T cells by Ia-expressing L cells has been documented. However, this antibody also prevents responses of L3T4-positive T cells to concanavalin A (Con A) when Ia$^-$ L cells are providing accessory function, which may indicate that Ia is not the target of L3T4 binding, as has been suggested. Second, antibody against LFA-1, which blocks virtually every other interaction between T cells and target cells, does not interfere with the activation of cloned T cells by L-cell transfectants, whether assayed by bulk proliferation, interleukin 2 (IL-2) production, or IL-2 receptor upregulation (36). Thus, either L cells overcome a normally required LFA-1:APC interaction using a distinct molecule, or the LFA-1 antibody inhibition of T-cell stimulation by antigen-presenting cells may involve something other than simple interference with cell adhesion through LFA-1:APC ligand binding.

Significant progress has been made by using the L-cell system to correlate Ia structure with T-cell recognition. We recently showed that the β^k_1:[β_1, TM, IC]^d exon-shuffled gene product, paired with A^k_α, not only is serologically indistinguishable from wild-type I-A^k, but also is responded to as if it were the wild type by all I-A^k restricted T cells tested to date (26). Although they remain to be confirmed with additional shuffles of other haplotype origin, these results suggest that the protein variations concentrated in the amino terminus of class II molecules play a predominant role in T-cell recognition.

This conclusion has been confirmed and extended by a second approach. We recently cloned and sequenced the A^k_β gene from a mutant B-cell hybridoma immunoselected with antibody against I-A (37). This A^k_β gene has a single nucleotide substitution at position 67 of the $A\beta_1$ domain, leading to a glu→lys exchange. The importance of this single β_1 mutation in both the serology and T-cell function of this gene was shown by creating an exon-shuffled gene of mutant L and β_1 with [β_2, TM, IC]^d and demonstrating that L-cell transfectants expressing this gene with A^k_α reproduce the mutant serologic and functional phenotype. A second independent mutant A^k_β gene has been cloned, and preliminary analysis suggests that another point mutation in the same region of $A\beta_1$ is responsible for altered immune reactivity to this gene product. The analysis of further mutants of this type, together with additional gene shuffling and mutagenesis experiments *in vitro,* should extend these initial observations on the regions of Ia which are important in T-cell recognition.

Discussion

The results described above point out the rapid progress made in the past few years in understanding the organization of class II (Ia) genes and the fine structure of their respective proteins. Nucleotide sequence analysis first indicated an amino-terminal concentration of the amino acid substitutions potentially critical to generation of the intraspecies polymorphic epitopes detected by antibodies and T cells. Gene transfer experiments, especially those involving L-cell fibroblasts as recipient cells, have demonstrated an unexpectedly strong influence of these polymorphic regions on the α:β pairing essential to membrane Ia expression. Although such asymmetry in chain pairing for Eβ:Eα had been reported (31), there was a general belief that "free" mixing of Aα and Aβ usually occurred in F_1 cells (32). Our results, although not yet including explicit tests of competition between products of two allelic β genes for a single α, suggest that such hybrid molecules will show wide variation in surface density depending on the haplotypes involved, and that biologically significant levels of hybrid or transcomplementing Ia molecules will only occasionally occur. Allele-specific effects on expression of these molecules may have important implications for understanding why certain DR heterozygotes often have a higher risk of autoimmune diseases than homozygotes.

Studies of gene transfer to L cells also revealed the existence of "forbidden" isotypically mixed (Aβ:Eα) molecules. This process also seems to be strongly influenced by NH_2-terminal allelic polymorphisms. The functional significance of such molecules is unknown, and additional experimentation is needed to determine whether there are also isotype-specific influences on Ia assembly, as most previous biochemical analyses suggest.

Functional studies with B lymphomas, and especially L-cell transfectants, have begun to dissect out the portions of Ia molecules important in T-cell corecognition of antigen plus Ia. In particular, the β_1 domain was found to be the necessary and, in the structural context of a complete Ia molecule, sufficient portion of the A_β^k molecule to permit T-cell recognition. Protein variation in the remainder of the β chain did not appear critical to such recognition in the one case examined, but more extensive tests of the generality of this result are needed. This conclusion from exon-shuffling experiments is supported by data involving Ia mutants. Not only the naturally occurring bm12 mutant Aβ gene (33) but also two independently derived mutant A_β^k genes differ from their wild-type counterparts by one or a small number of substitutions in the aa65–70 region of the β_1 domain. Significant alteration in T-cell recognition of the Ia molecules produced by these mutant Aβ genes was found. These results not only provide additional evidence for the critical importance of the β_1 domain as a whole, but also support the view that one (or more) of the hypervariable regions defined by allelic sequence comparisons will be key regions in the function of the Ia molecule with respect to T-cell recognition. Studies with genes generated from portions of the β_1 (or α_1) exons of allelic pairs or created by site-directed mutagenesis will refine these early conclusions.

The Ia-expressing L cells have also permitted study of the general mechanism of interaction of T cells with antigen-presenting cells. Antibody blocking experiments with anti-L3T4 and anti-LFA-1 reagents raised questions about the postulated role of these accessory molecules in T-cell function. From the available data it is not possible to formulate a unique hypothesis to explain the results, but it seems prudent to reexamine the possibility that antibody binding to these structures may directly affect steps in T-cell activation, perhaps in addition to effects on cell adhesion events.

It seems fair to say that the approach to the study of Ia structure-function relationships outlined here has yielded substantial early benefits. Future applications of recombinant DNA and gene transfer technology to the issues of control of Ia gene expression, molecular assembly, and T-cell recognition should rapidly provide a new level of understanding of class II genes and the role of their products in immunity.

Summary

A structure-function analysis of class II major histocompatibility complex molecules was carried out by using gene cloning and DNA-mediated gene transfer techniques. Nucleotide sequencing revealed that class II genes encoding Ia polypeptides have intron-exon organizations that reflect their protein domain structure. Amino acid polymorphism among allelic forms of Aα, Aβ, and Eβ genes is concentrated in the NH_2-terminal domains and in three or four hypervariable regions within these domains.

Studies of the expression of class II genes after their transfer into Ia^- L-cell fibroblasts showed unexpected asymmetries in assembly and/or expression of haplotype-matched (e.g.,

A^d_β:A^d_α) pairs compared to haplotype-mismatched (e.g., A^k_β:A^d_α) pairs. The quantitatively lower level of expression with mismatched pairs at a given level of Aβ and Aα mRNA could be partially compensated for by increased gene transcription. In addition, no absolute barrier to *inter*-isotypic chain pairing could be demonstrated, since A^d_β:$E^{a/k}_\alpha$-transfected cells expressed surface Ia with Aβ and Eα serologic epitopes, and both polypeptides could be coprecipitated with reagents specific for I-A or I-E.

The L cells expressing transfected Ia gene products were found to function as antigen-presenting cells to T-cell clones and hybridomas. Such L cells actively process complex antigens in a manner qualitatively similar to but apparently less efficient than processing by conventional accessory cells (e.g., macrophages). Although the L3T4 T-cell membrane antigen seems to function normally when these Ia^+ L cells are used as antigen-presenting cells, the LFA-1 molecule does not, since antibodies against LFA-1 fail to block interaction of T cells with the L-cell transfectants.

With this model it has been possible to show that the polymorphic NH_2-terminal domain of Aβ plays a critical role in the formation of serologic or T-cell-recognized epitopes of I-A. This was accomplished by employing a recombinant Aβ gene containing the first domain of A^k_β and the remainder of A^d_β. Further evidence for the importance of this region in T-cell recognition was obtained by sequencing two new mutant A^k_β genes, both of which have single amino acid changes in the third hypervariable region of the first domain and both of which show differences from the wild type in T-cell recognition of Ia plus antigen.

These results illustrate the utility of a combined molecular-cellular approach to the correlation of structural features of Ia with Ia function in T-cell recognition of antigens. They also indicate unforeseen complexities in the manner of formation and expression of Ia dimers, a finding with important implications for understanding histocompatibility-linked disease states.

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Major Histocompatibility Complex Class II Antigens: Genes and Proteins

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The phenomenon of graft rejection led to the discovery of the molecules we now call histocompatibility antigens. Graft rejection may be a very old phenomenon phylogenetically, and there is evidence that it can occur in both sponges and tunicates. In higher animals graft rejection was first studied in the 1930s by Peter Gorer in England (1) and by George Snell in the United States (2). They discovered a single region, which they called the H2 region, which is now known to encode a variety of genes involved in graft rejection. Since the pioneering studies of Gorer and Snell a number of other species have been examined, and in every case a similar genetic region has been identified. This region is now called the major histocompatibility complex, or MHC. In humans the MHC is located on chromosome 6 and in the mouse on chromosome 17. It contains three classes of genes, all involved in immune defense in one manner or another. Thus, the phenomenon of graft rejection, which is not a natural biological phenomenon in higher animals, provided a window into extraordinarily important and previously unknown aspects of immune defense mechanisms.

At the telomeric side of the MHC are located the class I genes, called HLA-A, -B, and -C in humans and H2-K, -D, and -L in the mouse (Fig. 1). (An exception is one of the mouse genes, H2-K, which appears to have been transposed to the centromeric end of the cluster at some time during the evolutionary development of the mouse.) At the centromeric side of the MHC cluster are located the class II genes, the HLA-D genes in humans and the I region genes in the mouse. Although the I region in the mouse was originally divided into five subregions by immunogenetic techniques (3), more recent molecular genetic data indicate that this region contains only two subregions, which are called I-A and I-E (4). In humans at least three subregions have been defined: SB, DC, DR (now DP, DQ, DR). Class III genes are the genes which encode some components of the complement system; they are located in the middle of the MHC in a region which was originally identified in the mouse and called the S region.

Class II Genes and Antigens

Class II genes are composed of two glycoprotein chains, a heavy or α chain and a light or β chain (Fig. 2). Each of these chains contains two domains external to the membrane: α1 and α2 in the heavy chain, β1 and β2 in the light chain (5). The two domains proximal to the membrane, α2 and β2, are both typical immunoglobulin (Ig)-like domains. The major polymorphic region is in the β1 domain. Both chains have a small transmembrane polypeptide, and both span the membrane and have small intracytoplasmic regions. The class II molecules are very similar in structure to the class I molecules, and both belong to a family of molecules which are made up of immunoglobulin domains. In addition, Thy1, a thymic protein in the mouse, is made of a single immunoglobulinlike domain (6). Immunoglobulin G (IgG)

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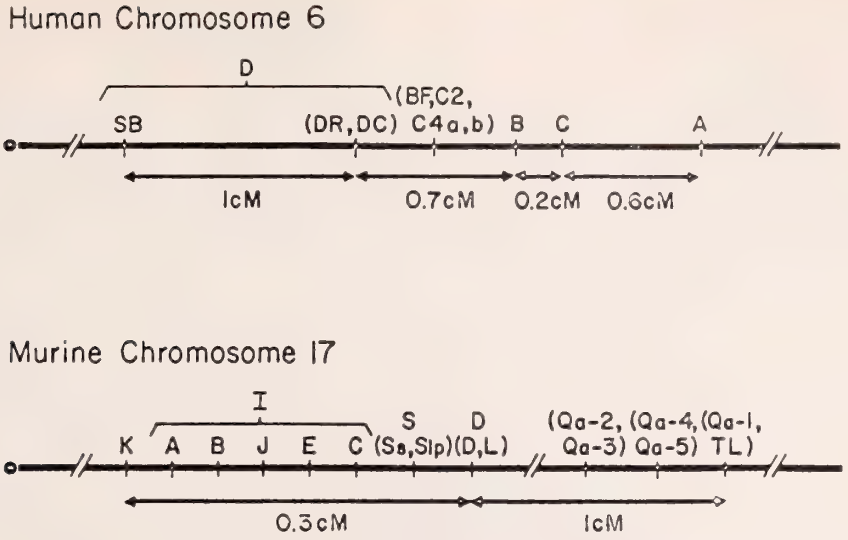

Human Chromosome 6
D
(BF,C2,
SB
(DR,DC) C4a,b)
B
C
A
IcM
0.7cM
0.2cM
0.6cM
Murine Chromosome 17
I
K
A
B
J
E
C
S
(Ss,Slp)
D
(D,L)
(Qa-2,
Qa-3)
(Qa-4,
Qa-5)
(Qa-1,
TL)
0.3cM
IcM

Fig. 1. Genes of human and murine major histocompatibility complex.

is made up of 12 such domains, and immunoglobulin M (IgM) of 14. Two additional molecules can be added to this family: the T-cell receptor (7), which has four immunoglobulinlike domains and is much like a class II antigen in structure, and the poly-immunoglobulin receptor in intestinal epithelium, which is built up of five immunoglobulinlike external domains (8).

An important complexity of the class II antigens is that they comprise several subsets. These subsets were first defined by the reactivity of alloantisera and were separated when monoclonal antibodies became available. The DR subset was separated with the monoclonal antibody L243 and the DC1 subset with the monoclonal antibody Genox 3.53 (9). The DR subset is itself complex, having at least two light chains, and can be further separated by making use of the specificity of the monoclonal antibody LKT111 to remove one of the DR subsets followed by L243 to remove the other (10). It is possible that an additional subset of DR antigens may be present since, besides the two clearly different amino-terminal sequences of the major light

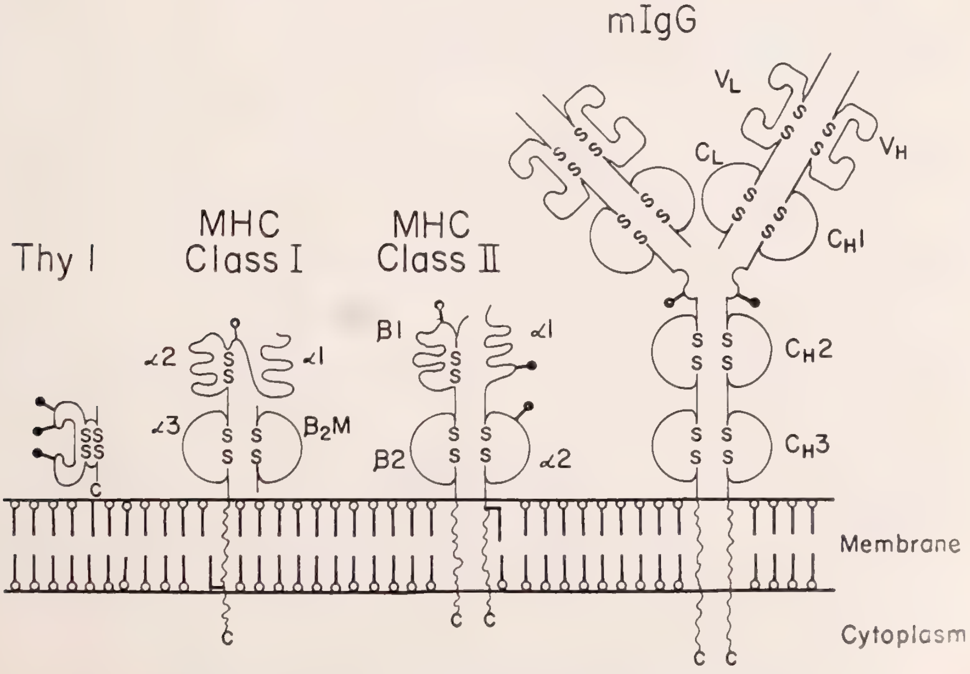
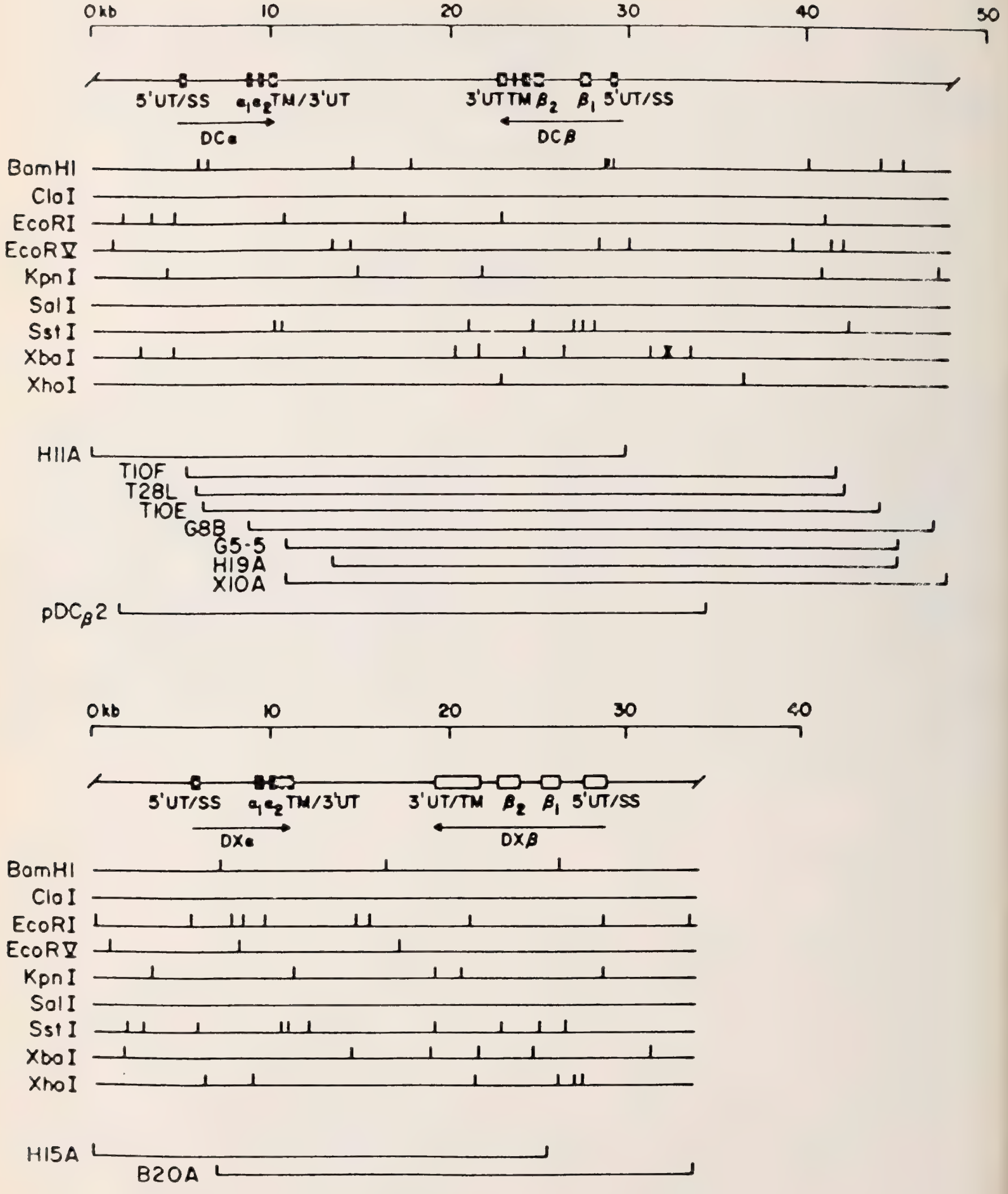
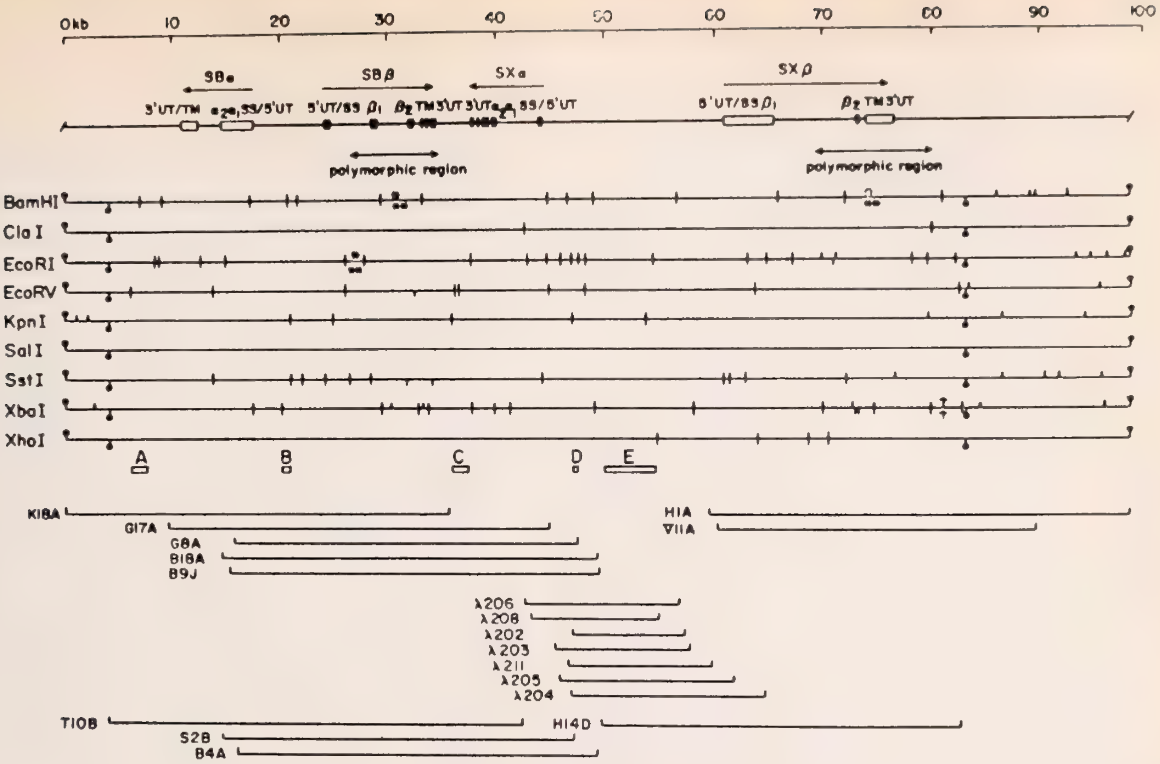

mIgG
V_L
V_H
C_L
C_H1
C_H2
C_H3
Thy I
MHC Class I
MHC Class II
α2
α1
α3
B₂M
B1
α1
B2
α2
Membrane
Cytoplasm

Fig. 2. Some proteins composed of immunoglobulin-like domains.

Fig. 3. Map of DC subregion of class II genes in humans.


0kb
10
20
30
40
50
5'UT/SS
α1α2TM/3'UT
3'UTTM β2
β1
5'UT/SS
DCα
DCβ
BamHI
ClaI
EcoRI
EcoRV
KpnI
SalI
SstI
XbaI
XhoI
H11A
T10F
T28L
T10E
G8B
G5-5
H19A
X10A
pDCβ2
0kb
10
20
30
40
5'UT/SS
α1α2TM/3'UT
3'UT/TM
β2
β1
5'UT/SS
DXα
DXβ
BamHI
ClaI
EcoRI
EcoRV
KpnI
SalI
SstI
XbaI
XhoI
H15A
B20A


0kb
10
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50
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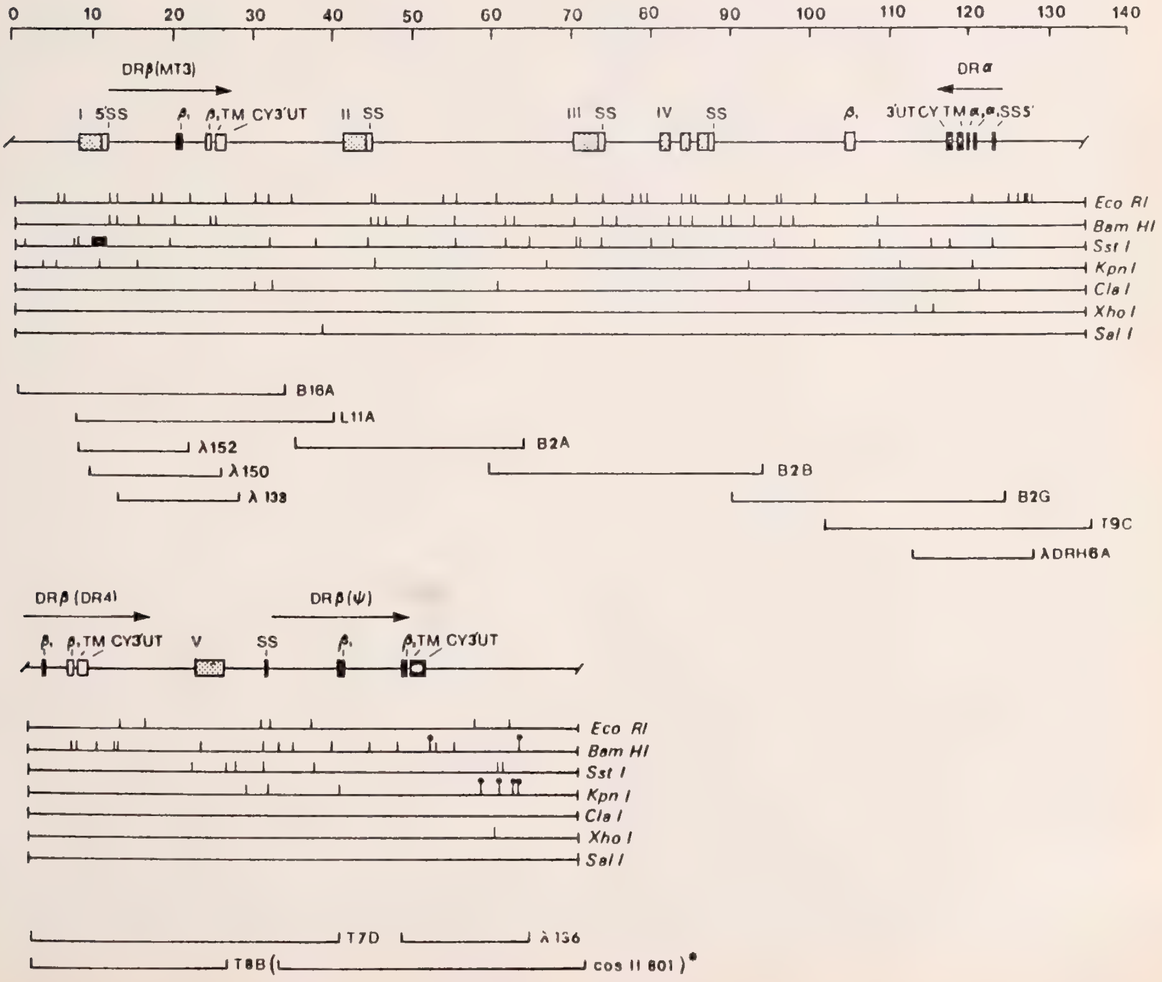
70
80
90
100
SBα
SBβ
SXα
SXβ
3'UT/TM
α2α1SS/5'UT
5'UT/SS β1
β2 TM 3'UT
3'UTα2α1 SS/5'UT
5'UT/SS β1
β2 TM 3'UT
polymorphic region
polymorphic region
BamHI
Cla I
EcoRI
EcoRV
KpnI
SalI
SstI
XbaI
XhoI
A
B
C
D
E
K18A
G17A
G8A
B18A
B9J
H1A
VIIA
λ206
λ208
λ202
λ203
λ211
λ205
λ204
T10B
S2B
B4A
H14D

Fig. 4. **Above** Map of SB subregion of class II genes in humans.
Fig. 5. **Below** Map of DR subregion of class II genes in humans.


0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
DRβ(MT3)
DRα
I 5'SS
β1
β2TM CY3'UT
II SS
III SS
IV
SS
β1
3'UT CY TM α2α1 SS5'
Eco RI
Bam HI
Sst I
Kpn I
Cla I
Xho I
Sal I
B16A
L11A
λ152
λ150
λ138
B2A
B2B
B2G
T9C
λDRH6A
DRβ (DR4)
DRβ (ψ)
β1
β2TM CY3'UT
V
SS
β1
β2TM CY3'UT
Eco RI
Bam HI
Sst I
Kpn I
Cla I
Xho I
Sal I
T7D
λ136
T8B
cos II 801)*

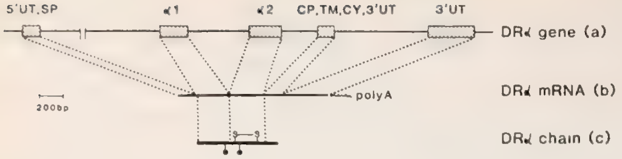


Fig. 6. Structure of the DRα chain gene, mRNA and protein.

chain proteins, a third DR-like light chain was identified in the same cell line, LB, by a DNA clone isolated from a complementary DNA (cDNA) library. However, although the LB cell line is DRw6 homozygous, it is not a consanguineous line, and thus the interpretation of these data is somewhat difficult. It seems obvious, however, that at least two subsets of DR antigens are expressed at the cell surface.

Additional information has come from gene cloning. We and others have made considerable progress toward establishing a physical map of the MHC region to correlate with the immunogenetic map. Three sets of cosmid clones have been obtained, including the DR, DC, DX, and SB subregions. However, these subsets have not yet been linked, and thus it is possible that there are still additional genes in this region. As far as we know at present, there are two DCα-like genes, DCα itself and DXα, and two DCβ-like genes, DCβ and DXβ (11) (Fig. 3). Similarly, in the SB subregion there are two α genes, SBα and SXα, and two β genes, SBβ and SXβ (12) (Fig. 4). We know that SXα and SXβ, at least, are pseudogenes in two haplotypes, but it remains to be shown whether functional genes may occur at these loci in other haplotypes. It is clear that the DXα and DXβ genes represent a structurally intact gene pair, but no evidence for the expression of this gene pair has been obtained so far (11, 13). In the DR subregion one α gene and three β genes have been identified, but the occurrence of three additional promoter-leader sequences in this subregion suggests that more genetic information could be present (14) (Fig. 5). Besides the five α chain genes mentioned above (DCα, DXα, SBα, and DRα), a sixth α gene, called DO or DZ, is known from the work of English (15) and Japanese (16) scientists, but we do not know yet where to place it in the physical map of the class II region. Similarly, evidence for an eighth β chain gene was recently presented in abstract form (C. Tonelle and E. Long, unpublished observations). Thus, there are at least six α chain genes and eight β chain genes in the human genome.

A very detailed genetic map of the SB subregion was recently obtained (12). Two different haplotypes, SB3 and SB4, were identified. Two interesting points emerge from this work. First, the genes in the SB subregion are oriented head-to-head rather than tail-to-tail as they are in all the other subregions. Second, the genetic region is highly conserved in exons as well as in introns, except within small polymorphic regions of both β chain genes. The class II region of the human MHC is much larger than that of the murine MHC. Only two α chain genes and at most five β chain genes have been identified in the murine I region. Considerable gene expansion in this region occurred in humans subsequent to the human-mouse divergence. It is, of course, tempting to relate these findings to some typical features of the human species—for instance, to attribute the greater longevity or variety of habitat of humans to the fact that we may be able to make much finer immunological responses than other species.

A typical class II gene is organized in such a way that the exons which encode the gene faithfully represent structural domains of the protein. The signal peptide is encoded in the first exon, the α1 or β1 domain in the second, the α2 or β2 domain in the third, and the transmembrane and cytoplasmic regions in the fourth (Fig. 6). Southern blots can also provide some idea of the number of genes present in different haplotypes. Southern blots of homozygous DR1 to DR7 cell lines probed with a DRβ cDNA clone under high-stringency conditions show that there are three separate sequences encoding DRβ genes in most haplotypes. In addition, DR4 and DR7 give very similar Southern blots; DR3, DR5, and DR6 give similar Southern blots; but DR1 and DR2 appear to be distinct (17). This pattern of similarity, in fact, corresponds to the MT serological specificity pattern, and we saw it earlier in our studies of the β chain proteins but were uncertain of its significance. In these earlier experiments the β1 (amino terminal) and β2 (carboxy terminal) domains of these antigens could be separated by chymotryptic digestion. The same pattern was observed for DR3, 5, and 6 and for DR1, 4, 7, and 8, while DR2 was different from any of these. This, too, is reminiscent of the MT serological classification (18).

Expression of Class II Genes

To investigate the expression of the DR antigens from the three β chains in the DR subregion in more detail, monoclonal antibodies were employed. Monoclonal antibodies LKT111 and L227 recognized two distinct subsets of DR antigens in DRw6 cells, both of which were also recognized by L243. Recently, 109d6 was shown to be a monoclonal antibody directed against the MT3 specificity (19) which is present on DR7 cells. Indeed, 109d6 precipitates a molecule with a β chain which is distinct from the other β chains precipitated by the DR-specific monoclonal antibodies, being more acidic and having a slightly lower apparent molecular weight. On the other hand, LKT111 and L243 precipitate identical material. Monoclonal antibody L227 is more complex and has not yet been fully analyzed. It appears to recognize both a DR subset and the DC subset on DR7 cells (20). Thus, an epitope normally present on DR molecules appears to be present on DC molecules in DR7 cells. This phenomenon of epitope shuffling, as it may be called, illustrates one of the problems of attempting to obtain monoclonal antibodies for class II antigens in humans. Similar experiments were carried out with DR4 cells, and again LKT111, L227, and L243 all recognized the single DR subset present in these cells while 109d6 recognized the MT3 subset, which had a clearly distinct β chain but the same α chain as the DR molecules (17). Genomic blots suggest that two DCβ genes and two SBβ genes are present in the genomes of a number of homozygous cell lines. One of them, the SXβ gene, is polymorphic with *Hind* III. Another fragment obtained by *Hind* III digestion is not polymorphic with this enzyme, but is polymorphic when other enzymes are employed.

A very important question is: What are the rules for chain association? Is each α chain gene able to combine only with its own small family of β chain genes; or are closely related α chain genes, such as SBα and SXα or DCα and DXα, able to combine with the same family of β chain genes; or finally, is every α chain gene able to combine with every β chain gene? At present we do not know the rules for chain association, and the serological reagents that are available, alloantisera and monoclonal antibodies, are of no help in approaching this problem. In fact, the most complex alloantisera and monoclonal antibodies, which have been discarded because of their very complexity, could conceivably be very valuable in providing answers to these questions.

Expression of class II genes can be studied both in vivo and in vitro. In vivo, they have been studied by activation with γ interferon (21). Under the influence of γ interferon change the morphological phenotype of cultured endothelial cells changes dramatically. Seven class II antigen genes are activated: the α and β genes of the DR, SB, and DC subsets and the gene for the γ or invariant chain, which is encoded on a different chromosome (chromosome 5). There is an enormous induction of messenger expression by γ interferon, and it is paralleled by a similar increase in surface antigen expression, which can be monitored with the appropriate monoclonal antibodies. The appearance of messages specific for DR and DC is followed by the appearance of surface antigen. In vitro genes can be transfected into recipient cells. Cosmid clones containing SBα and SBβ genes were transfected into two types of cells: the P3M mouse macrophage line and the LTK$^-$ mouse fibroblast line (12). The transfectants were analyzed with the SB-specific monoclonal antibodies Tu39 (22) and MHM4 (23). Mouse L cells transformed with the SB cosmid and analyzed by FACS showed a Tu39-positive population which expresses the SB antigen. In the case of the mouse macrophage line, the extent of expression as monitored with Tu39 was quite low, and to amplify it we resorted the brightest 5% of cells and obtained a population of cells with a high level of expression of SB antigen. This population was grown up and employed for immunoprecipitation with SB-specific monoclonal antibody. The immunoprecipitation monoclonal antibody gave a more basic β chain and more acidic α chain (12). The pattern was complex and its elucidation requires further analysis. These experiments illustrate the importance of monoclonal antibodies to specific class II subsets in the analysis of phenomena observed with transfected genes.

Applications of DNA Probes

Several practical applications of the DNA probes generated in the course of these studies have become apparent. In the first place, polymorphisms can be detected at the DNA level (by Southern blots) just as they can be detected serologically. For example, the polymorphism of the heavy chain of the DC antigens is readily detected by this method. Other polymorphisms are more difficult to define with the probes available, although they can be detected. As better probes become available, it should be possible to type individuals more accurately at the DNA level than is presently possible serologically. In addition, the probes have been used in hybridization experiments in situ to localize several genes on human chromosome 6 rather precisely. Moreover,

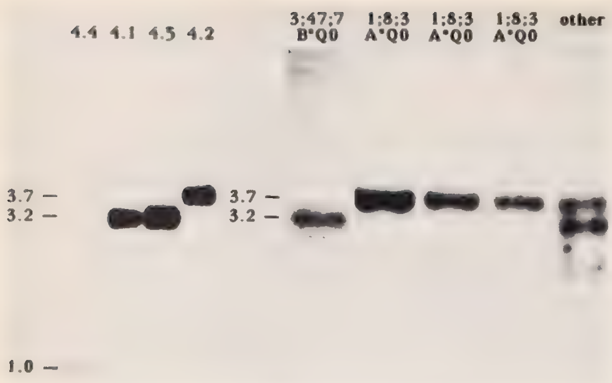


Fig. 7. Hybridization of human 21-steroid hydroxylase cDNA clone to cosmid clones encoding C4A (4.1 and 4.5), C4B (4.2), to DNA of a patient with 21-steroid hydroxylase deficiency (3;47;7:B*Q0) and to normal human DNA (other). Data from White et al. 1985 (26).

they have been used to study a rearrangement of chromosome 6 involving a reciprocal translocation of chromosome 6 with chromosome 14 and to define the precise break point in this translocation. It is likely that with the use of these probes much more accurate diagnoses of genetic abnormalities in humans will be possible in the future. These probes are also being used to study possible polymorphisms in HLA-linked autoimmune diseases such as multiple sclerosis, juvenile onset diabetes, and rheumatoid arthritis.

An extremely important recent development is the use of cosmids encoding the class III genes, components of the complement system in the middle of the human MHC, to detect a genetic defect in congenital adrenal hyperplasia. This disease is due to a deficiency of 21-steroid hydroxylase. A cDNA clone corresponding to this enzyme was obtained and used as a probe. Two genes for steroid 21-hydroxylase, flanking the complement C4A and C4B genes, were detected. One of these is deleted along with the C4B gene in homozygous congenital adrenal hyperplasia, and the other is deleted along with C4A gene in a phenotypically normal individual (Figs. 7 and 8).

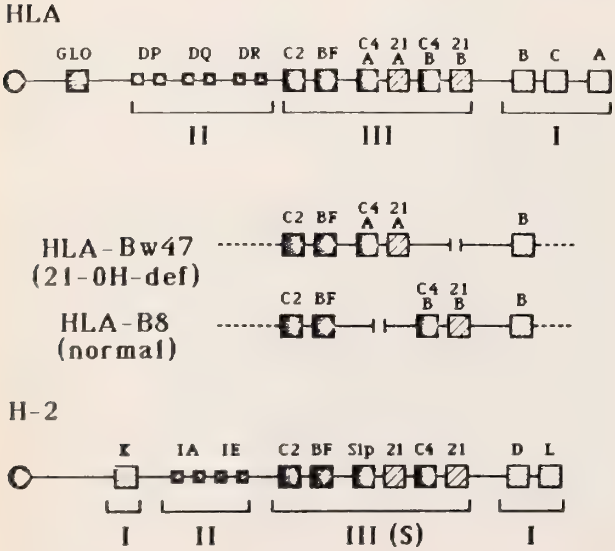


Fig. 8. Class II region of human MHC. Deletions of C4A or C4B together with a 21-steroid hydroxylase gene (26).

A full list of references can be found in the papers cited above and in two recent reviews (24, 25).

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Surface Receptors Involved in Human T Lymphocyte Activation

ELLIS L. REINHERZ, M.D.

Although the molecular mechanisms that underlie antigen recognition in humoral immunity have long been understood, until recently the means by which cytotoxic and immunoregulatory T lymphocytes recognize antigen have been elusive.

T lymphocytes, unlike B lymphocytes, predominantly recognize antigen when it is associated with membrane-bound products of the major histocompatibility complex (MHC) (1, 2). This "dual" recognition is important for activation of both cytotoxic effector T cells and immunoregulatory T cells. T cells of the former category lyse specific target cells, including cells infected with virus that display viral antigens, whereas immunoregulatory T cells act as inducers or suppressors for interactions among T cells, B cells, macrophages, and other cells.

The exquisite specificity of T cells was thought to be determined by unique, clonally distributed (clonotypic) cell surface receptors. The ability to propagate human T lymphocyte clones in vitro has now made direct recognition of these clonotypic determinants possible. With use of T cell clones as immunogens, murine monoclonal antibodies specific to single clones have been generated and used to define a novel class of clonotypic molecules found on the surface of all T cells (3). Just as monoclonal immunoglobulins have been employed to characterize antibody structure, monoclonal T-cell populations have provided the cellular material with which to elucidate the structure of the receptor for antigen and MHC gene product.

Human T-Cell Receptor Structure and Function

Studies using cloned antigen-specific T lymphocytes and monoclonal antibodies directed at their various surface glycoprotein components have led to identification of the human T-cell receptor as a surface complex comprised of a clonotypic 90KD Ti heterodimer and the three monomorphic T3 molecules (gamma, delta, and epsilon) (Fig. 1). The gamma subunit has an apparent molecular weight of 25KD and the delta and epsilon subunits have each apparent MW of 20KD (4, 5). Both gamma and delta have N-linked glycosylation sites. All five T-cell receptor subunits are transmembrane proteins.

Upon binding to the surface of a clone, monoclonal antibodies to either the T3 components or their associated unique Ti heterodimer induce rapid loss of the T3-Ti complex, a process termed modulation, and they inhibit all antigen-specific functions of a given clone. However, at the same time, these antibodies produce a markedly enhanced clonal proliferative response to IL-2. The latter is a 15KD sialoglycoprotein that interacts with activated T cells through specific membrane receptors distinct from the T3-Ti complex and is known to induce T-cell growth upon surface binding (6). Moreover, when coupled to the surface of a solid support such as Sepharose, the appropriate surface-linked anti-Ti and anti-T3 antibodies were able to induce IL-2 secretion and clonal proliferation, analogous to the physiologic ligand (that is, antigen) itself. These findings suggested that clonal proliferation resulting from triggering of the T3-Ti antigen receptor might be mediated through the growth factor IL-2.

To investigate some of the molecular mechanisms underlying T-cell proliferation after triggering of antigen receptors, we utilized a variety

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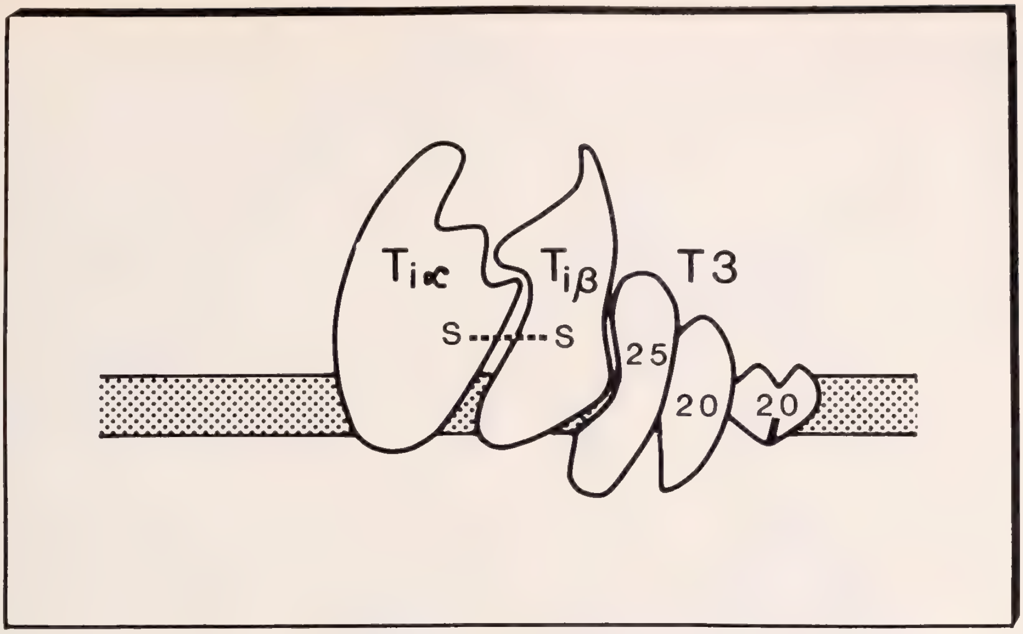

Fig. 1. Subunit composition of human T-cell antigen receptor. Ti alpha and Ti beta subunits are held together by S-S bonds and are associated with 25KD chain of T3 molecule. Alpha and beta subunits are anchored in cell membrane with their transmembrane segments. T3 complex consists of two additional subunits with molecular weights of 20KD.

of antigen-specific human T-cell clones, in conjunction with anti-clonotypic, anti-IL-2 receptor and anti-IL-2 monoclonal antibodies (7). The results clearly demonstrated a functional linkage between antigen receptor and IL-2 receptor on all clones tested. Specifically, soluble anti-T3 or anti-Ti antibodies, like physiologic ligand, induced a sixfold increase in IL-2 receptors. Moreover, when these antibodies were bound to Sepharose beads, they induced IL-2 secretion and clonal proliferation analogous to antigen. That the latter response was mediated by IL-2 was evident from the finding that either monoclonal anti-IL-2 receptor or anti-IL-2 antibodies blocked clonal proliferation following T3-Ti triggering.

The induction of IL-2 receptors occurred as a discrete event in and of itself and did not appear to require Ti crosslinking (or more precisely, crosslinking of more than two receptors, if one assumes that the antigen receptor is univalent) because it could be mediated by binding of soluble anti-Ti monoclonal antibody to the clone's surface. In contrast, production and release of IL-2 and subsequent clonal proliferation did not occur in the absence of apparent receptor crosslinking initiated by surface-bound antigen on the stimulator cells or anti-Ti coupled to Sepharose. The identity of the phenotypic changes induced by anti-Ti anti-receptor antibodies and appropriate combinations of nominal antigen and MHC has provided further support for the notion that the Ti complex is the receptor for both antigen and MHC.

It thus appears that T-cell proliferation occurs as a series of complex and precisely orchestrated events (Fig. 2). Resting T-cell clones express few or no IL-2 receptors but display a maximal number of surface antigen receptors. However, T3-Ti receptor triggering by antigen plus MHC gene products of the appropriate haplotype or Sepharose-bound anti-clonotypic monoclonal antibodies results in modulation of the T3-Ti complex, thus diminishing the number of surface antigen receptors and rapidly inducing surface IL-2 receptor expression. Furthermore, such activation also leads to endogenous IL-2 production, secretion, and subsequent binding to IL-2 receptors on the same clones. Once a critical number of IL-2 receptors have bound IL-2, DNA synthesis and cell mitosis occur. Finally, in the absence of continued antigenic stimulation, there is re-expression of the surface T3-Ti antigen receptor complex and reciprocally, a reduction in the number of IL-2 receptors.

In the case of resting T lymphocytes, the same scheme applies but macrophage and/or IL-1, in addition, is essential for responsiveness to T3-Ti receptor triggering (8). Whether the observed ability of IL-2–dependent T-cell clones to proliferate following T3-Ti triggering in the absence of exogenous IL-1 is secondary to an activation state beyond that requiring IL-1 is unknown,

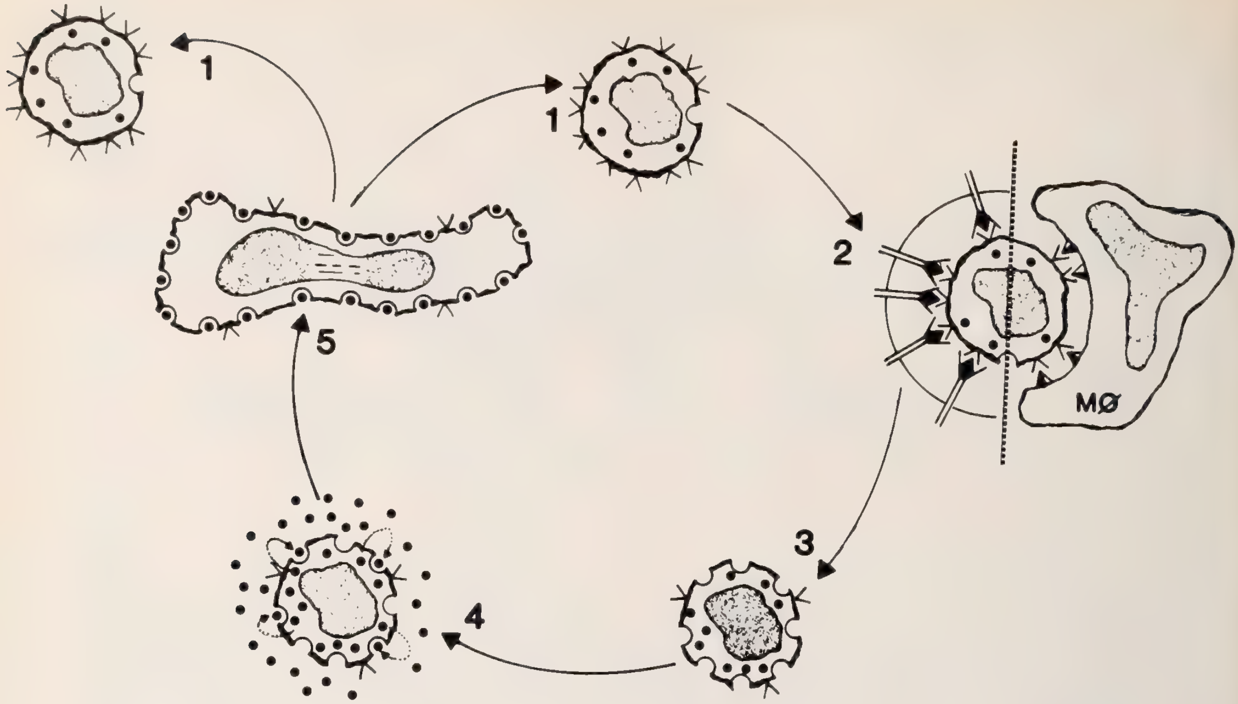

Fig. 2. Schematic outline of T-cell proliferation mediated by interleukin-2–dependent pathway. Numbers 1–4 depict stages in IL-2–dependent pathway. Stage 1 shows T cell in resting state with few or no surface interleukin receptors (*u* shaped) and maximal number of antigen receptors (*v* shaped). Stage 2 shows T3-Ti triggering by a macrophage (MØ) with antigen and major histocompatibility complex restricting element (half-filled triangles) or surface bound antibodies to T-cell receptor (filled symbols with stems), resulting in modulation of T3-Ti and induction of IL-2 receptor expression (stage 3). Stage 4 shows production and secretion of IL-2 (black dots) which binds to its receptors. In stage 5, critical density of occupied IL-2 receptors is achieved and DNA synthesis and mitosis occur. Finally, in the absence of additional antigenic stimulation, T3-Ti receptor complex is re-expressed (stage 1).

but clearly appears likely in view of the fact that T cells do not produce IL-1.

The present set of findings is in agreement with recent studies with lectin-stimulated peripheral blood T lymphocytes in which it was shown that IL-2 receptor expression occurred during G1 phase of cell cycle and preceded DNA synthesis (9). Moreover, the correlation of IL-2 receptor levels with the proportion of cells in active cycle emphasized the importance of antigen receptor triggering on IL-2 responsiveness, especially when IL-2 concentrations become limiting. In addition, induction if IL-2 receptors by antigenic stimulation as shown here provides a rational basis for the empiric observations that IL-2–mediated clonal expansion in vitro is facilitated by repetitive antigenic stimulation.

The view that is emerging regarding the relationship of the T3-Ti antigen receptor to the IL-2 receptor points to a mechanism whereby external stimuli, that is, antigen, direct the magnitude and extent of T-cell clonal proliferation by means of the IL-2 hormone-receptor system. Immunologic specificity is ensured by the antigen dependence of the IL-2 receptor expression, and yet the reciprocal appearance of T3-Ti and IL-2 receptors presumably leaves the cell in a state of responsiveness to either the hormone or antigen ligand. However, the transient expression of the IL-2 receptors themselves serves as a fail-safe system to avoid the possibility of uncontrolled growth through an IL-2–dependent mechanism. Alterations of this mechanism are already becoming apparent in the clinical setting, so that further information about these features of T-cell growth and interaction with putative oncogenes and/or their products needs to be subject to critical evaluation.

Human T-Cell Activation via T11 SRBC Binding Protein

In addition to the T3-Ti receptor complex, recent studies have indicated that an unrelated 50KD surface glycoprotein, originally defined as the sheep erythrocyte binding protein, can serve to activate T lymphocytes (10). On the basis of both qualitative cellular distribution studies and competitive blocking experiments, three distinct T11 epitopes termed T11$_1$, T11$_2$, and T11$_3$ could be defined on this structure by means of monoclonal antibodies. T11$_1$ is associated with the sheep erythrocyte binding site on the 50KD molecule. Monoclonal antibodies to this epitope could block rosette formation between sheep red blood cells and human T lymphocytes. In contrast, antibodies to the $T11_2$ and $T11_3$ epitopes did not block sheep erythrocyte rosetting. Moreover, the $T11_3$ epitope was expressed on activated but not resting T lymphocytes or thymocytes. Nevertheless, the $T11_3$ epitope was localized to the same molecule as $T11_1$ and $T11_2$. Thus, each of the monoclonals reacted with a single 50KD structure, as shown by sequential precipitation studies and peptide map analysis of individual 50KD molecules isolated with either anti-$T11_1$, anti-$T11_2$, or anti-$T11_3$ antibodies.

Analysis of $T11_3$ epitope expression indicated that it was one of the earliest determinants to appear following human T-cell activation, and was expressed within 24 hr of phytohemagglutinin or antigen stimulation and prior to expression of transferrin receptor. Moreover, binding of antibody to $T11_2$ and $T11_3$ epitopes was mitogenic for resting T cells and T-cell clones in the absence of IL-1 or accessory cells and induced IL-2 secretion and regulatory lymphokine production. Thus, for example, anti-$T11_2$ plus anti-$T11_3$ triggering of antigen-specific MHC restricted inducer T-cell clones resulted in clonal proliferation, IL-2 production, and activation of their inducer program resulting in B-cell IgG secretion. This latter result suggests that both BCGF and BCDF activities were elicited. That anti-$T11_1$ neither alone nor in conjunction with anti-$T11_3$ could induce T-cell activation equivalent to anti-$T11_2$ plus anti-$T11_3$ makes clear, in retrospect, why the process of sheep erythrocyte rosetting was not associated with activation of T lymphocytes.

The macrophage and IL-1 independence of the mitogenic effect of soluble anti-$T11_2$ plus anti-$T11_3$ antibodies contrast with the cited findings with antibodies to T3-Ti. Moreover, the requirement for both anti-$T11_2$ and anti-$T11_3$ in the induction of proliferation is clearly due to more than crosslinking alone since, even in surface-bound form, neither anti-$T11_2$ Sepharose nor anti-$T11_3$ Sepharose alone induced proliferation.

The observation that triggering of $T11_2$ plus $T11_3$ epitopes concurrently resulted in T-cell proliferation and IL-2 production suggested that these two phenomena were related. This relationship was confirmed in functional studies where it was shown that antibodies to the IL-2 receptor blocked clonal proliferation to exogenous IL-2 as well as mitosis induced by anti-$T11_2$ and anti-$T11_3$.

It therefore appears that the resting T cell can be activated by either of the two T lineage-specific surface structures. The antigen receptor pathway is initiated via antigen/MHC or surface-bound anti-Ti or anti-T3 antibody (that is, a crosslinkable form of the antibody), whereas the alternative pathway of T-cell activation is mediated by anti-$T11_2$ plus anti-$T11_3$. In addition, recent studies show that a 10KD lymphokine, termed IL-4, is produced by T3-Ti crosslinking of T4+ helper T cells and is the natural T11 ligand (11) (study by C. Milanese, N. E. Richardson, and E. L. Reinherz). Both T3-Ti and T11 triggering results in T-cell activation through an IL-2–dependent pathway. In either case, functional output includes clonal proliferation, IL-2 receptor expression, IL-2 secretion, and regulatory function. The capacity of antigen-triggered helper T cells to activate other resting target T cells via T11 with the IL-4 lymphokine in a fashion independent of the specific antigen receptor structures on those target populations may be important in providing a mechanism for cellular recruitment, thus augmenting the immune response in the absence of further antigenic stimulation.

Thymic Differentiation

The differentiation of eukaryotic cells which leads to the acquisition of their specialized functions involves precisely orchestrated changes in gene transcription and translation. Initiation of such events often requires a specific inductive microenvironment. In the development of T lymphocytes, for example, immunoincompetent hematopoietic precursors (bone marrow or fetal liver cells) enter the subcapsular cortex of the thymus, where they undergo a series of changes in cell surface glycoproteins and only then acquire a functional T-cell repertoire. Subsequently, they are disseminated into the peripheral lymphoid compartment.

In humans, discrete stages of intrathymic ontogeny have been defined on the basis of monoclonal antibody probes directed at unique T-lineage-specific surface glycoproteins (Fig. 3) (11a). Thus, the earliest identifiable T-lineage cell expresses the sheep erythrocyte binding protein, T11, but no other T-lineage-specific markers (T11+T6−T4−T8−T3−) (stage I). With further maturation, the TL-like molecule T6 is acquired and T-lineage-specific T4 and T8 molecules are coexpressed to varying degrees on the same cell (T11+T6+T4+T8+T3−) (stage II). Both stage I and stage II thymocytes are located in the cortex of the thymus and represent ~10% and 60%–70%

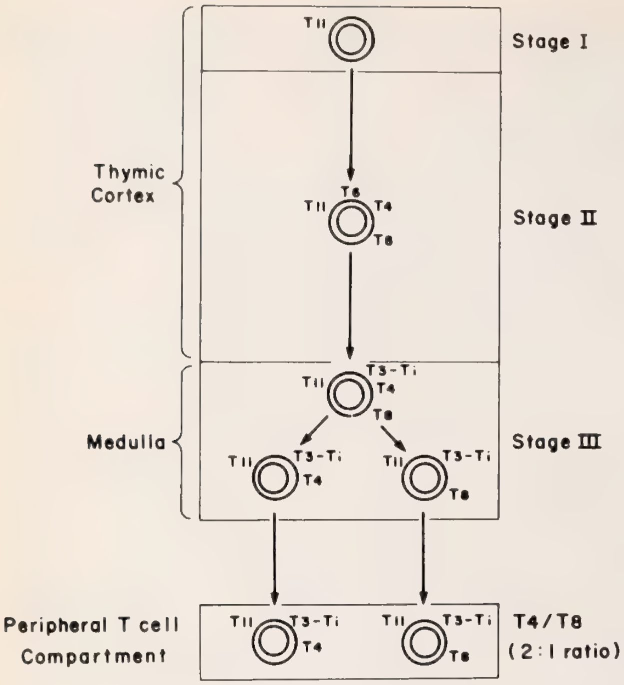

Fig. 3. Stages of intrathymic ontogeny.

of the total thymocyte population, respectively. With even further maturation, the expression of the cortical-specific marker T6 ceases, the T3 glycoproteins are acquired, and thymocytes are driven to terminally differentiate in one of two directions; either the T4 molecule is maintained and the T8 molecule lost or, reciprocally, T8 is maintained and T4 is lost (T11+T6−T4+T8−T3+ or T11+T6−T4−T8+T3+) (stage III).

Although stage III thymocytes represent only a fraction (<30%) of thymocytes, they alone demonstrate antigen responsiveness and hence are functionally competent. The structural basis for this association has recently been elucidated, since it has been shown that the T3 molecules represent monomorphic subunits of the T cell receptor for antigen. To define the activity of Ti genes during intrathymic ontogeny, a number of laboratories have isolated and sequenced full-length cDNA clones encoding the Ti alpha and beta subunits (12). It has been found that the human Ti beta gene specifically rearranges in T lineage cells and that this rearrangement predominantly occurs at the level of the stage II thymocytes (13). Transcriptional analysis of Ti alpha and beta genes indicates that Ti beta RNA transcription is evident in the stage II compartment, whereas Ti alpha transcription is not fully active until stage III (14). Furthermore, surface expression of the T3-Ti molecular complex is restricted to the more differentiated stage III thymocyte compartment. These findings imply that rearrangement of T cell receptor genes precedes surface receptor protein expression analogous to activation and expression of genes encoding surface immunoglobulin on B lymphocytes. The absence of the Ti beta gene rearrangement in stage I thymocytes provides an ideal cellular compartment with which to begin to analyze the basis of the gene activation process.

Although both T3-Ti and T11 pathways of T cell activation can apparently be utilized by mature T lymphocytes, only the T11 structure is expressed at the level of stage I or stage II thymocytes. In this regard, it has recently been shown that the combination of anti-$T11_2$ and anti-$T11_3$ antibodies or the IL-4 lymphokine can trigger stage I and II thymocyte activation and induction of IL-2 receptor expression within the T3-thymocyte compartment (15). Evidence in support of this view has been provided by three lines of data: (a) anti-T11 antibodies or IL-4 can induce thymocytes to express IL-2 receptors as judged by reactivity of monoclonal antibodies specific for the human IL-2 receptor in indirect immunofluorescence; (b) these anti-IL-2 receptor antibodies will precipitate a 60KD glycoprotein from anti-$T11_{2\text{-}3}$ induced thymocytes that is indistinguishable from the IL-2 receptor isolated from activated peripheral blood T lymphocytes or T-cell clones; and (c) such thymocytes proliferate to purified IL-2 in the absence of other lymphokines such as IL-1. Media or antibodies specific for the T6, T4, T8, or T1 glycoproteins have no such effects. In contrast to their effects on peripheral blood T lymphocytes, however, anti-$T11_{2\text{-}3}$ antibodies do not induce thymocyte proliferation without exogenous IL-2. Presumably the IL-2 gene cannot be activated in the majority of these lineage precursors. That a small percentage of stage I and II thymocytes express $T11_3$ epitopes and IL-2 receptors implies that the T11 pathway functions in vivo.

These data suggest that the T11 molecule may be an important structure by which the thymocyte program is activated prior to surface T3-Ti receptor expression. That this molecule is the earliest T lineage structure to appear in ontogeny and is strongly conserved throughout phylogeny would further support this view. It is probable that IL-4 is produced within the thymus and that this ligand is mimicked by the combination of anti-$T11_2$ and anti-$T11_3$ antibody. A second important feature of the T11 pathway worthy of reemphasis is that activation in this system is macrophage− and IL-1−independent. Given the fact that IL-1 is presumably present in limiting amounts within the thymus, this characteristic may be of great functional significance during intrathymic ontogency.

These studies provide considerable insight into pathways of human T lymphocyte activation without elucidating early molecular events involved in the process of signal transduction. In this regard, considerable progress has been forthcoming in this field (16–22, and unpublished study by A. Alcover, C. Milanese, and E. L. Reinherz, "Role of Na+/H+ exchanger in the activation of human lymphocytes"). The data (not summarized here) indicate that Ca2+, pH, and protein kinase C all play pivotal roles in early activation events.

Summary

The T lymphocyte receptor for antigen and histocompatibility molecules is a molecular complex comprised of five polypeptide chains. Both the 49KD alpha and 43KD beta chains are immunoglobulin-like and thus contain variable domains responsible for ligand binding. In contrast, the 20-25KD T3 gamma, delta, and epsilon chains are monomorphic structures, presumably involved in transmembrane signalling. The alpha and beta subunits are disulfide-bonded to each other and held in noncovalent association with the T3 chains. T3-Ti receptor crosslinking leads to conformational modification of a second T-lineage-specific molecule, termed the 50KD T11 structure, and leads then in turn to protein kinase C activation, elevation in intracytoplasmic free calcium, and Na+/H+ antiport stimulation.

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Immunoglobulin Genes and the c-*myc* Oncogene in Plasmacytomagenesis

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Great progress has been made in defining the genetic and structural basis of immunoglobulin (Ig) biosynthesis and the origin of antibody diversity. In B lymphocytes a novel mode of gene differentiation takes place: discontinuous coding sequences on a chromosome bearing an immunoglobulin gene are spliced and rejoined to form functional transcriptional units for immunoglobulin light and heavy chains. This process involves joining of structural genes in the variable (V) region to J_L-C_L genes for light chain rearrangements or to D and J_H-C_H genes for heavy chains. The V_H-D-J_H-C_H complex can undergo another form of rearrangement, immunoglobulin C_H switching, in which one V_H-D-J_H-C_H rearranged DNA is uncoupled from the C_H gene and rejoined to another C_H gene downstream. Differentiated B lymphocytes undergo coordinate developmental and physiologic changes to expand clonal populations appropriately during stages of immunoglobulin gene differentiation and to distribute cells of a differentiated clone strategically in the organism. Thus, controls of cellular proliferation probably also change during B-lymphocyte differentiation.

It is generally thought that immunoglobulin-secreting cells have a short half-life in the body (1) and are ultimately eliminated by emerging into a postmitotic state, that is, maturing to the plasma cell stage. It is clear that some normal, relatively immature immunoglobulin-secreting (plasma) cells are capable of division (1). Very mature plasma cells have pyknotic nuclei. Plasmacytoma cells are another example of replicating differentiated cells. The inducible plasmacytomas of the mouse, which have been so important in unraveling the complexities of immunoglobulin biosynthesis, offer another challenge—that of explaining in biochemical and genetic terms the basis for their abnormal growth. This complex problem involves understanding the process of development of plasma cell tumors.

A key set of facts makes the mouse system particularly intriguing. First, plasmacytomas, which are uncommon neoplasms in the mouse, can be induced in high frequency (about 60%) in inbred BALB/c mice by intraperitoneal implantation or injection of nonmetabolizable substances, such as solid plastics or mineral oils (2). Second, BALB/c and NZB mice are susceptible to plasmacytoma induction, but most other common inbred strains (C3H, C57BL, DBA/2, A, CBA) are resistant (3, 4). These two facts suggest that specific genes in BALB/c mice have actions that influence the probability of neoplastic transformation in immunoglobulin-secreting cells. Third, the inducing agents are not direct mutagens. For example, the alkane pristane (2,6,10,14-tetramethylpentadecane) cannot be metabolized. Pristane preparations obtained from commercial sources contain contaminants that may be biologically active but are probably not responsible for the plasmacytomagenic properties of the compound (5). It is assumed that almost any of the molecular types of the many alkanes found in medicinal light-white mineral oils would induce plasmacytomas. A common property of the different agents that induce plasmacytomas is their capacity to evoke a chronic inflammatory response. Fourth, 98% of the plasmacytomas of the BALB/c mouse have a common cytogenetic abnormality—chromosomal translocation or deletion involving the D2 band region of chromosome 15 (6).

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Chromosomal Translocation and c-*myc* Activation

Early prebanding cytogenetic studies of mouse plasmacytomas by Yosida and co-workers revealed that primary BALB/c plasmacytomas are highly aneuploid (7). When banding techniques came into use, studies of long-term transplanted plasmacytomas showed the presence of t12;15 translocations in several tumors, suggesting nonrandomness (8, 9) of this translocation. The significance of t12;15 became apparent in a cytogenetic study of seven primary tumors, which showed that all of them had reciprocal translocations of either chromosomes 12 and 15 (rcpt12;15) or chromosomes 6 and 15 (rcpt6;15) (10). Since that report, 46 primary or early transplantation generation plasmacytomas of BALB/c or BALB/c congenic origin have been studied (Table 1), and it has been found that rcpt12;15 occurred in 67% and rcpt6;15 in 21% of them (6). Other variants (including deletion of D and E bands in chromosome 15) were found in the remaining tumors, and only one tumor, ABPC 22, lacked a cytogenetic abnormality of chromosome 15 (11). The break points in chromosome 12 involved the immunoglobulin heavy chain locus, and the band involved in chromosome 6 occurred in a region compatible with a location near the gene complex of the immunoglobulin kappa light chain.

The consistent involvement of band D2 on chromosome 15 aroused considerable interest in the genes located near this break point. Harris et al (12) studied the process of immunoglobulin C_H switching in plasmacytomas and found Ig C_H sequences joined to segments of DNA that did not originate from chromosome 12. Some of these non-immunoglobulin-associated rearranged DNAs (NIARD) were cloned and found to hybridize with DNA originating from chromosome 15 (13). A giant step was then taken by Shen-Ong, Cole, and their associates, who hypothesized that the NIARD sequence might be an oncogene (14). Influenced by the promoter insertion model of Hayward et al. (15) for activation of *myc* oncogenes in bursal B-cell B lymphomas of the chicken, Shen-Ong et al hybridized avian c-*myc* with mouse plasmacytoma DNA and found that c-*myc* was rearranged in the plasmacytomas (14). They went on to show that the chromosome 12–15 junction was, in fact, a joining in reverse transcriptional order of the immunoglobulin switch region to c-*myc* (Fig.). Extensive studies with many plasmacytomas, particularly by Adams, Bernard, Cory, and Gerondakis (16–18), have shown that the rcpt12;15 translocation usually involves different parts of Sα or Sμ (and more rarely one of the Sγ sites). The Sα is involved even in tumors that do not secrete immunoglobulin A (IgA) (K. B. Marcu, personal communication).

In all vertebrates studied so far, the c-*myc* gene has three exons: E1, E2, and E3 (Fig.). Exons E2 and E3 code for the *myc* protein, which has a calculated molecular weight of 45,000 but migrates in polyacrylamide gels as a protein of molecular weight 64,000 to 68,000 (19). The E1 exon and its flanking sequences contain regulatory sequences: three DNA-ase I sites 5′ to E1 (20), promoters P1 and P2 at the beginning of E2, and cryptic promoters in intron 1 (21). These regulatory sequences appear to control transcription positively and negatively.

The break point in the c-*myc* oncogene can occur in a multiplicity of sites between the 5′ end of c-*myc* and the beginning of E2 (16–18). The t12;15 translocational process in effect separates the coding from the regulatory sequences of the gene, and this process appears to modify the controls of c-*myc* transcription. At this time there is not universal agreement on the critical physiological effect of the translocation. Early experimental evidence supported the notion that normal c-*myc* transcription was activated only by an inductive process in a specific stage of the cell cycle, but this point is now debated. More recent studies of several different "normal" cell lines have shown that transcription of c-*myc* and synthesis of *myc* protein were constitutive throughout the cell cycle (22, 23). It is likely that the deregulation of c-*myc* resulting from the t12;15 translocation has a more subtle explanation and must be interpreted in relation to differentiation-specific events in B lymphocytes. Further, it is clear that c-*myc* transcription can be induced (24). Thus, it will be important to define more precisely the effects of different levels of *myc* transcription and *myc* protein on the physiology of B-lymphocyte proliferation. Activation and increased transcription of c-*myc* may be essential in bringing B cells from a true G0 stage to a G1 stage in the mitotic cycle.

In normal B-lymphocyte development, most mature immunoglobulin-secreting cells are destined by an unknown process to cease dividing and die. In fact, immunoglobulin-secreting plasmacytoma cells are extremely fragile and die very rapidly in vitro if deprived of essential growth factors (25). This indicates, at least for

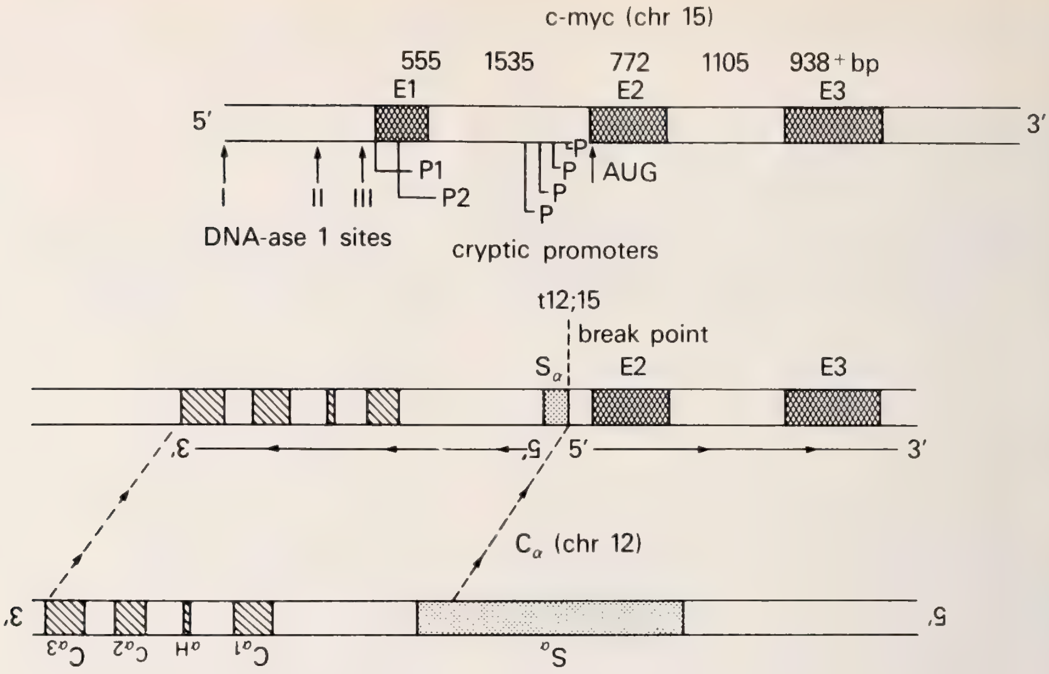

Fig. Disruption of c-*myc* oncogene in t12;15 translocation.

plasmacytoma cells, that the maintenance of competence for cellular mitosis depends on strong exogenous influences. One can speculate that an important effect of deregulating the c-*myc* gene is an increased level of c-*myc* transcription and that this might have the effect of maintaining competence for cellular proliferation. Deregulation of c-*myc* alone may not be sufficient to deregulate the controls of B-cell proliferation to the point of autonomy, but the continuing cell divisions render the cells vulnerable to new mutational events.

The rcpt6;15 translocational process does not result in the rearrangement of c-*myc*. The break point is thought to be many kilobases (about 72 kb) distant from the 5′ end of c-*myc* (26). The break in chromosome 6 occurs in a 1- to 3-kb region 5′ to C_K (26). At present it is not clear how the physiology of the c-*myc* gene is disturbed in 21% of the mouse plasmacytomas with rcpt6;15.

Origin of the Translocations and DNA Strand Breaks

A major question that remains is why nonrandom translocations involving immunoglobulin genes and the c-*myc* locus occur with such regularity. It is now clear that is not an exceptional characteristic of the mouse plasmacytoma. Homologous translocations have been found in Burkitt's lymphoma in humans (18) and in spontaneously developing rat immunocytomas (plasmacytomas) (27) (Table 2).

Breakage of DNA strands in the immunoglobulin complex loci could occur in specific sites during differentiation, mainly the 3′ end of V genes or the 3′ and 5′ ends of genes in the J_H, J_L, or D region during the V-J joining process or switch region (S) sites during immunoglobulin C_H switching. It is tempting to suggest that during these processes "loose ends" of DNA are available. Indeed, the most common forms of translocation involve switch sequences (16–18). A few J_H break sites have also been found (18). However, a substantial number of translocations in chromosome 6 are not easy to explain by a loose-end hypothesis.

A recently discovered locus on chromosome 15 called pvt-1, located 72 kb 5′ of c-*myc*, is the site involved in many of the rcpt6;15 translocations (26, 28). Why breakage occurs in pvt-1 during rcpt6;15 is not known. Moreover, the chromosome 6 breakage which occurs 5′ to C_K has not been clearly related to the process of V-J joining (26). Several hypotheses have been advanced to explain the breaks in chromosome 12 and joining to chromosome 15 in rcpt12;15 translocations; one is that certain sequences recognized by switch recombinases are located near c-*myc* and allow these enzymes to coordinately join an immunoglobulin locus with c-*myc* (29). Some additional support for this possibility comes from the

TABLE 1

Chromosomal Translocations in Plasmacytomas

rcpt12;15	t(inv12);15, 12q	rcpt6;15	del 15	None
CBBTEPC 1	CBPC112	CBBTEPC1	ABPC26	ABPC22
CBBTEPC6	ABPC17	CBBTEPC3		
CBBTEPC8		CBBTEPC8		
CBBTEPC9	t12;15, 12q			
	ABP45	ABPC4		
CBPC49(λ)		ABPC20		
		ABPC103		
TEPC1072(λ)		ABPC105		
TEPC1081(λ)				
TEPC1082(λ)		invRb6;15		
TEPC1094(λ)		CAKTEPC1198		
		CAKTEPC1199		
TEPC1004		CAKTEPC2016		
TEPC1010				
TEPC1017				
TEPC1033				
TEPC1165				
ABPC18				
ABPC24				
ABPC33				
ABPC47				
ABPC48				
ABPC52				
ABPC60				
ABPC65				
ABPC72				
ABPC89				
CAKTEPC1192				
CAKTEPC2001				
CAKTEPC2002				
CAKTEPC2003				
CAKTEPC2004				
CAKTEPC2005				
CAKTEPC2006				
Frequency	34/46 (74%)	10/46 (21%)	1/46 (2%)	1/46 (2%)

Adapted from references 6, 10, 11.

fact that immunoglobulin A is the predominant heavy chain class in BALB/c plasmacytomas (3, 29) and the Sα site is the switch site most frequently involved in rcpt12;15 translocation.

If a specific recombinase does participate in IgA switching, it might be involved not only in switching the productively rearranged chromosome 12 but also in joining an available Sα region on chromosome 12 to chromosome 15. An intact germline Sα region is only infrequently involved in the translocational process (17). Although the translocated chromosome 12's are not active in immunoglobulin heavy chain synthesis, some have undergone nonproductive immunoglobulin V_H-D-J_H rearrangements. DNA sequences or secondary structures near the c-*myc* locus recognized by the recombinase have not been defined, although several sequences have been proposed; see, for example, (29). Chromosomal breaks often appear to occur in vulnerable sites (called fragile sites) in the genome; about 50 of these have been described in humans (31), none so far in the mouse. It is possible that a large region near c-*myc* is a fragile site, but this remains to be demonstrated. The hypothesis usually advanced to explain nonrandom translocations states that other translocations do occur, but go undetected because they are not associated with a growth advantage and competence to continue cycling. B cells with random translocations would be eliminated from the system.

Hypothetical Generator of Chromosomal Breaks

The metabolic inertness of pure alkanes, mineral oils, and plastics indicates that they are not direct clastogenic agents; however, they may

TABLE 2

Homologous Forms of Nonrandom Chromosomal Translocations Involving Immunoglobulin Loci and the c-myc Locus

		Chromosomal translocations related to immunoglobulin complex loci		
Species and (c-*myc* location)	Ref.	Heavy chain	Kappa light chain	Lambda light chain
Humans (chromosome 8)	40	t8;14	t8;2	t8;22
Mouse (chromosome 15)	6	rcpt12;15 tinv12;15;12q	rcpt6;15	Not found
Rat (chromosome 7)	27	rcpt6;7*		

* Probable assignment of immunoglobulin H in the rat is to chromosome 6.

indirectly stimulate inflammatory responses in accessory cells that can generate clastogenic products. Interperitoneal pristane and mineral oils are emulsified into tiny oil droplets by the motions of the gut. These droplets are then phagocytosed by free macrophages and neutrophils, and many of the oil-laden cells adhere to peritoneal surfaces, where they form a chronic inflammatory tissue that is vascularized from beneath and covered with mesothelium. This tissue has both acute (neutrophilic) and chronic (macrophagic) inflammation. The oil granulomatous tissue also contains many lymphocytes and scattered plasma cells, and within a few weeks foci of proliferating plasma cells can be seen. These foci often contain atypical plasma cells that stain intensely. The morphology of these plasma cells is not unlike that of the cells that populate fully developed plasmacytomas. Thus the foci may represent a preplasmacytoma stage (33).

The inflammatory cells are potential generators of mutagenic or clastogenic substances (32), including oxygen radicals that are produced during the phagocytosis of oil. Oxygen radicals produced within or secreted by neutrophils or macrophages can attack the plasma membrane lipids of nearby B lymphocytes and induce peroxidation of the phospholipids in B-cell membranes. Peroxidation of polyunsaturated lipids can be a chain reaction that generates new lipid radicals and fragmentation products of lipid peroxidation. These intracellular compounds are potentially capable of attacking DNA.

This hypothetical chain of events has not yet been proved by the identification of intermediates or responses in B cells. However, some recently obtained indirect evidence from this laboratory supports this general hypothesis (33). The nonsteroidal anti-inflammatory drug indomethacin, when it is administered daily to BALB/c mice in their drinking water (20 μg/ml), dramatically inhibits plasmacytoma formation in the mice. Indomethacin does not prevent the development of the oil granuloma tissue (33); thus it must be blocking the formation of inflammatory products that influence the development of plasma cell tumors. Although the primary pharmacologic effect of indomethacin is inhibition of prostaglandin synthetase (cyclooxygenase), there is evidence that the drug inhibits radical formation in accessory inflammatory cells, thereby diminishing the load of radicals to which B lymphocytes are exposed. Workers in several laboratories have demonstrated that indomethacin inhibits chemiluminescence produced by neutrophils (34–36). Indomethacin does not appear to inhibit the growth of primary plasmacytoma cells in pristane-conditioned syngeneic hosts (37).

Other mutagenic two-cell systems have been described. Emerit and Cerutti (37) have cultivated human lymphocytes with accessory cells (probably neutrophils and macrophages), activated the cultures with phorbol esters, and observed clastogenic effects in the lymphocytes. Recently, Weitzman et al. (38) induced transformations in C3H 10T ½ fibroblasts by exposing them to activated human neutrophils. In epidermal carcinogenesis, the inflammation inducing phorbol esters which promote tumor development appear to do so by stimulating accessory cells to produce radicals. Indomethacin is an inhibitor of epidermal tumor promotion (39).

Thus, an attractive hypothesis for plasmacytomagenesis is that the chronic inflammation is the source of mutagens that potentially can mutagenize B lymphocytes that have entered the oil granuloma. Since the B cells appear to be stimulated to proliferate and differentiate in this microenvironment, probably by growth factors supplied by the inflammatory cells, they are more vulnerable to neoplastic transformation.

Summary

Plasmacytomas induced in the mouse provide an intriguing model for the pathogenetic events that transform normal plasma cells to an autonomous proliferative state. Key genetic lesions are the nonrandom translocations rcpt12;15 and rcpt6;15, that involve the D-band region of chromosome 15. The selective involvement of immunoglobulin gene loci in the translocations remains only partially explained. Factors that may promote translocations have been discussed. It has been proposed that radicals are liberated by the inflammatory process caused by the attempts of the body to contain the oil (pristane) by phagocytosis and that these radicals are potential clastogens that increase the chances for chromosomal breaks to occur. Indirect evidence to support this possibility come from the fact that the nonsteroidal anti-inflammatory agent indomethacin inhibits plasmacytomagenesis. The translocations occur within the c-*myc* oncogene (that is, the rcpt12;15 translocation) or distantly as in the rcpt6;15 translocations. The effect of the translocations is generally thought to be deregulation of the c-*myc* oncogene.

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Transgenic Mice in Immunology

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Transgenic mice are animals into which foreign, molecularly cloned DNA has been inserted. Because transgenic mouse production entails transfer of foreign DNA at the earliest stages of development, and because donor genes can be passed through the germline, this experimental system allows the investigator to study expression of isolated genes in the context of normal development of the organism. This powerful technology is particularly useful in immunology, where gene complexes and the cells in which they are expressed interact in a developmentally and spatially regulated way. Experiments demonstrating efficient expression of cloned immunoglobulin genes within cells of the immune system with the production of active proteins are reviewed here; also reviewed are studies indicating the feasibility of gene injection for therapy of immune deficiencies and technical problems encountered on using the transgenic mouse system, with suggested solutions.

History

Transgenic mice were first produced in 1980 (1). The successful introduction of foreign genetic material into animals was the culmination of several previous independent research efforts. In the early 1960s, chimeric, or "allophenic," mice were first produced (2, 3). With this technique, the zona pellucida of the 8-celled embryo is removed, after which 2 or more embryos of different genotype can be aggregated. After co-culture of the embryos, a single large blastocyst develops. When implanted, such blastocysts frequently develop into adult genetic mosaics (4). Although this technique does not constitute gene transfer into the organism, the ability to genetically mark subsets of cells in the embryos allows for tracing of cell lineages, study of sex determination, and a variety of other experiments, including the effect of normal cells on the neoplastic transformation of cell strains normally predisposed to oncogenesis (5). These animals were also used for immunologic studies which showed that the two contributing strains in chimeras retained their immunogenicity when used as donors in skin grafts, but that the allophenic mice were tolerant to grafts received from either strain used in construction of the chimera (5).

A significant advance in chimera construction was made when two groups observed that pluripotent teratocarcinoma cells, when injected into the mouse blastocyst, could become normalized and take part in the formation of a chimeric mouse (6, 7). That a cultured cell line was able to take part in normal development raised the possibility of genetically engineering the germlines of mice. For example, the cultured cells could be selected for genetic deficiencies, such as hypoxanthine-guanine phosphoribosyl transferase (HPRT), and then inserted into blastocysts. If the $HPRT^-$ cells contributed to the germ cell population of such a chimera, the sperm or egg which transmitted this deficiency could then give rise to a uniformly $HPRT^-$ animal (8). Another use of chimeras derived in part from teratocarcinoma lines was based on the finding that purified DNA could be introduced into cultured cells and express the genes contained within it (9). DNA-mediated gene transfer could theoretically be applied to teratocarcinoma cells, after which the transformed recipients could be introduced into chimeric mice (10). Transmission of teratocarcinoma cells through the germline could then lead to introduction of a foreign gene into every cell of the offspring. An important feature of both

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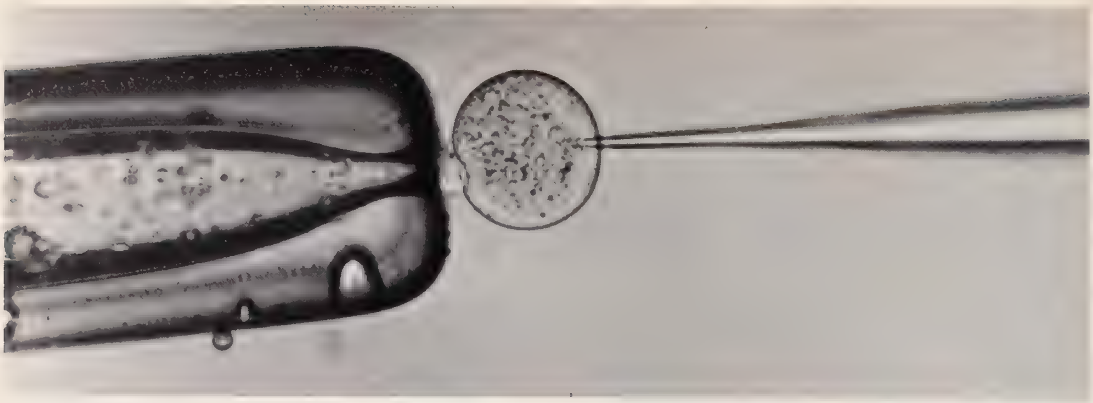

Fig. 1. Microinjection of DNA into mouse oocyte. Oocyte is fixed in position by suction applied to holding pipet (left); microneedle (right) is inserted into male pronucleus. Injection of from 100 to 30,000 copies of foreign DNA results in efficient production of transgenic mice.

strategies is their dependence upon transmission of teratocarcinoma derivatives through the germline of chimeras. Although such germline transmission has occasionally been reported (11, 12), it is simply too infrequent for practical use. Thus this strategy of gene transfer, which has many potential uses not provided by the transgenic mouse system, has not yet proved workable.

That genes could be introduced directly into the cleaving embryo was first suggested by experiments with viruses. SV40 DNA, when injected into the blastocyst cavity, was subsequently found in tissues of adult mice (13). In these experiments integration of the foreign genes could not be demonstrated, but subsequent studies with the Moloney murine leukemia virus (MoMuLV) showed that the proviral DNA of this RNA virus could integrate into a single site in the genome of the cleaving embryo and be transmitted through the germline as a Mendelian trait (14). Experiments with MoMuLV have proved of significant use in clarifying many problems in developmental genetics, but they have not provided an approach to insertion of genes other than those carried by the virus into mice.

The successful transfer of molecularly cloned DNA of any type was accomplished by microinjection of DNA (Fig. 1) into the pronucleus of the fertilized egg (1). With this approach, genes from any source could be injected at the earliest stages of development and could be found to integrate into the recipient genome and pass to succeeding generations of mice by breeding (15–17). The term “transgenic” for these mice was coined by Gordon and Ruddle (18).

A number of features of the transgenic mouse system proved remarkably fortuitous for studies of developmental gene regulation. First, the transformation frequency was often found to be quite high (16, 17, 19). Second, with only the rarest of exceptions (18, 20) the foreign genes gained access to the germline; and third, and perhaps most remarkably, genes were often expressed very efficiently (17, 21–24), and, in the case of immunoglobulin genes, with an efficiency apparently independent of the chromosomal integration site (25). Tissue specificity of expression appeared to be dictated by promoter and/or enhancer sequences close to the start site of transcription (23, 24, 26–31) and could function across species lines (21, 22, 24). Happily, efficient, tissue-specific expression appeared to be a particularly salient feature of cloned immunoglobulin genes (25, 32, 33). These observations made possible the exploitation of transgenic mice for genetic dissection of the immune system by transfer of subcloned fragments of the major histocompatability locus into an intact animal with a distinctly different genetic background than that from which the genes were cloned. The extent to which this application of the transgenic mouse system has been put to use is reviewed below.

Problems with the Procedure. Despite all positive features of this procedure, studies of foreign gene expression in transgenic mice can still present some problems. These difficulties stem from two main features of the system. First, genes are not often integrated as single, intact copies. Rather, they most frequently integrate as large, head-to-tail concatamers (16, 34). Efforts to study problems such as methylation can be hampered when multiple identical copies of a gene are present at a single chromosomal site (34). The other problem is that foreign gene conformation and expression is not always stable after transmission through the germline. Occasionally, large numbers of copies of genes in a head-to-tail concatamer may be lost, and this loss can be associated with changes in expression (34). In addition, the structure of the integrated unit may change during transmission through the germline. An example of foreign sequence instability is presented below. Experimental data which suggest a solution to the problem of concatameric integration in transgenic mice is also presented.

Efficiency of Expression of Immunoglobulin Genes

Light-Chain Genes. Brinster et al (25) microinjected a murine immunoglobulin κ light-chain gene cloned from a myeloma cell line into mouse embryos to produce transgenic mice. The injected gene had already undergone rearrangement and was functional in the parent cell line. This gene was chosen for injection because its variable portion is transcribed at exceedingly low levels in normal mice. As an internal control for RNA production, endogenous constant region transcripts were probed. Of 11 mice born from these injections, 6 carried the foreign gene, and one of those 6 had two integration sites.

Expression was evaluated by examining RNA from spleen, with liver used as a negative control. All expressed large amounts (approximately 3,000 copies per cell) of RNA in spleen; only low levels were detected in liver. The small amounts of RNA in liver were presumed to be due to contamination of liver tissue by circulating lymphocytes, a suggestion reinforced by follow-up study which directly correlated the level of expression in tissues other than spleen with the extent of B-cell contamination (32). Despite the fact that variable numbers of copies of the foreign gene were present among the lines, the independent integration sites in each line, and the presence of plasmid molecules on the injected material, all transgenic mice expressed approximately the same level of RNA in spleen. The injected gene thus carried sequences powerful enough to override all of these potentially inhibiting factors and to direct tissue-specific expression of rearranged immunoglobulins. Another interesting point was that failure to observe expression in liver showed that gene rearrangement is not the factor which determined the tissue distribution of immunoglobulin gene expression. Although the injected gene was already rearranged prior to differentiation of the liver, this organ was unable to express the gene.

Heavy-Chain Genes. Similar studies of trangenic mice injected as embryos with rearranged heavy-chain genes gave somewhat different results. Grosschedl et al (33) injected a functionally rearranged μ heavy-chain gene and studied 4 lines of transgenic mice which harbored between 20 and 140 copies per cell of the injected sequence. In addition to having been cloned and microinjected in rearranged form, this gene had the additional feature that its polypeptide product specifically binds the hapten 4-hydroxy-3-nitrophenyl (NP). Thus, production of a functional product could be measured by testing transgenic mouse sera for NP binding activity.

Although efficient expression of the foreign gene was observed in several cases, expression was more variable than that of rearranged light-chain genes. Examination of a variety of nonlymphoid tissues as well as macrophages revealed no expression except in heart muscle, where despite accurate assessments of B-cell contamination by measurements of constant region heavy-chain transcripts, the number of μ transcripts was clearly above background. High expression was also found in T lymphocytes, which were purified from B cells by removal of Ig positive cells followed by adherence of the remaining cells to petri dishes coated with T-cell-specific anti-Lyt 2 antibody. These experiments demonstrated only two-fold less transcription of the μ gene in T cells than in B cells.

To test for functional protein production from the foreign gene, sera of transgenic mice and unprimed negative controls were tested for NP binding activity. Substantial NP binding was detected in all transgenic mouse lines, though the amounts were quite variable. The degree of serum binding did not strictly correlate with RNA levels found in spleen. The mouse with the highest NP binding activity had fewer foreign gene transcripts two other animals.

These results contrast with those of rearranged light-chain gene injections in several important ways. First, expression is more variable in the animals injected with heavy-chain genes. Second, tissue-specific expression of the heavy-chain gene is not rigorously maintained in transgenic mice. T cells expressed high levels of the transcript and heart muscle expressed low but clearly detectable amounts. The expression of rearranged heavy chains in T cells was interpreted as indicating that gene rearrangement is the process which normally limits expression of this gene in lymphoid cells other than B cells. Normally, T cells do not express the V_H gene because it is not rearranged. In transgenic mice, where the gene is rearranged at conception, this barrier is bypassed and the T cell is able to express. Expression in heart muscle is harder to interpret, but it may take place because of the presence of embryologic commonality between heart and lymphoid cells, or the presence of trans-acting factors in heart nuclei which are able to stimulate transcription from the rearranged V_H gene promoter (33). These experiments also go on to demonstrate the production of functional immunoglobulin from injected gene, a fact which suggests that gene injection might be used for the genetic engineering of the immune response in animals.

Effect of Expression on Host Immunoglobulin Gene Rearrangement

In follow-up studies of transgenic mice carrying rearranged immunoglobulin light-chain genes, Ritchie et al (35) have examined the effect of foreign gene expression on host gene rearrangement. Normally, when a B-cell clone rearranges and expresses one of its 8 light-chain genes, the remaining genes are excluded from rearrangement and expression. This phenomenon of allelic exclusion is poorly understood, but is accessible to examination with transgenic mice injected with an exogenous, rearranged light-chain gene which is expressible.

This examination was made by producing a series of hybridomas from transgenic mouse lymphocytes. Both the foreign gene protein and RNA were distinguishable from the endogenous κ-chain transcript and protein product, and rearrangement of the endogenous light-chain gene could be readily assessed by restriction enzyme digestion and Southern blotting (35). The resulting hybridoma clones fell into three categories: those that secreted the foreign κ-gene product, those that secreted the endogenous product, and those that secreted no immunoglobulin.

When the transgenic κ chain was secreted, no endogenous light-chain product was found in the cytoplasm, and no endogenous light-chain rearrangement could be detected. When the free endogenous light chain was secreted, however, some hybridomas did also have transgenic κ chains in the cytoplasm, though these were never secreted. Secretion of endogenous light chains as part of a whole immunoglobulin molecule was always associated with failure of exogenous κ chain expression. When transgenic κ chains are present in the cytoplasm but not secreted, the endogenous gene is not expressed, but may be rearranged. These results can be summarized as indicating that expression of one rearranged light-chain gene is sufficient to shut off expression of the others but is not adequate to block rearrangement of others. It appears that rearrangement is blocked by the formation of a functional immunoglobulin molecule (35). These interesting data have contributed significantly to our understanding of allelic exclusion.

Correction of Immune Deficiency By Ia Genes

Le Meur et al (36) have injected Ia antigen coding sequences into mouse embryos and have examined the ability of such genes to alter the immune responsiveness of transgenic mice. The Ia antigens fall into two complexes, the A and the E. These gene products, inducible by activators such as γ-interferon, are presented to T cells in association with foreign antigens. Only if the T cell is able to recognize "self," in the form of the surface Ia molecule, is it then able to recognize the associated foreign protein as antigenic. Probably because of the strong selective pressure for exquisitely sensitive discrimination of self from foreign proteins, the Ia antigens are highly polymorphic. Some strains of mice lack the ability to express the E complex entirely. Because the expression of E-complex Ia antigens is necessary for generating an immune response to the synthetic polypeptide poly Glu-Lys-Phe (referred to as GLϕ), mice lacking an expressible E gene are unable to respond to this antigen.

Le Meur et al microinjected a cloned, expressible E gene into $H\text{-}2^{(bxs)}$ hybrid mice, which are unable to express E antigens because of a deletion in the 5′ region of their endogenous E gene. Two transgenic mouse lineages were established and tested for their responsiveness to GLϕ. Transcripts from the foreign gene were detected in both, and the tissue distribution of expression followed that of E transcripts in control mice. The inducibility of the foreign E gene in transgenic mice was tested by exposing peritoneal macrophages from both transgenic mouse pedigrees to $IFN\text{-}_{\gamma}$ in vitro. A significant stimulation was observed in both cases. To determine if this foreign gene could allow the transgenic animals to produce functional antibodies to E restricted antigen, transgenic mouse offspring were immunized with GLϕ and subsequently tested for antibody production. As controls, littermates not inheriting the foreign gene and unresponsive $H\text{-}2^{(bxs)}$ animals were primed. As positive controls, responsive $H\text{-}2^{(bxa)}$ mice were primed, and the ability of mice in the transgenic lines to mount a normal immune response was tested by priming with ovalbumin. Results showed a significant generation of GLϕ antibodies in the transgenic mice, with none in negative littermates. All controls worked appropriately. Thus, injection of a functional Ia antigen of the E complex was adequate to correct immune deficiencies in strains otherwise unable to express these genes. Similar results were simultaneously reported by Yamamura et al (37).

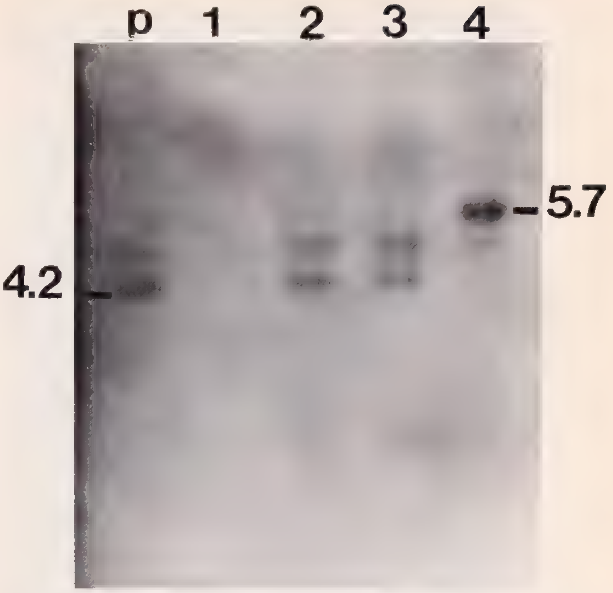

Fig. 2. Sequence instability in transgenic mice. Parental animal (p), has retained a single copy of a foreign plasmid, giving a 4.2 kb band after *Xba*I digestion and Southern blot hybridization. Of its 4 offspring shown, no. 1 has not inherited the new gene; nos. 2 and 3 have inheritied the sequence in apparently the same conformation as the parent. Offspring no. 4, however, has inherited the gene in altered form, the exogenous sequence appearing as a 5.7 kb band.

Technical Problems

Although the usefulness of transgenic mice for immunologic research is obvious, problems remain with this system which both complicate analyses of experiments and raise questions about the immediate application of this technology to genetic engineering of agricultural animals or human beings. These problems include the fact that the site of integration of foreign genes cannot yet be controlled, that expression of injected genes is often variable, that large concatamers containing multiple copies of an exogenous sequence are often integrated, and that the structure of integrated material may be unstable. Examples of these problems, their significance, and in one case, a possible solution, will be given below.

Random Integration of Foreign DNA. Three types of studies have indicated that the integration of foreign DNA into the mouse genome after microinjection is random and is not mediated by sequence homology between donor and recipient material. When DNA carrying a sequence which is highly repeated in the mouse genome is injected, its frequency of integration is no higher than that of a sequence with no homology to mouse DNA (1). When genes such as the globins are injected, in situ hybridization studies demonstrate that they do not insert at the mouse globin locus (16). When mouse metallothionein genes are injected, their integration does not alter the restriction map of the endogenous gene and thus does not take place at the site of the endogenous gene (34).

Random integration can be a problem for two reasons. First, the integration site in many cases does affect expression of foreign genes, and thus makes it difficult to predict efficient expression in any given case. Second, insertion of foreign DNA into an important host sequence can have untoward mutagenic effects. Insertional mutations have already been observed in several lines of transgenic mice (38–40). Until very recently there appeared to be no obvious strategy for solving this problem. However, a sensitive system for screening mammalian cultured cells for integration by homologous recombination has recently been developed by Smithies et al (41), who observed that 1/1000 of mammalian cells transfected with a plasmid linearized within a cloned β-globin gene integrated that gene at the endogenous β-globin locus. Although this finding is exciting, it is not feasible to produce 1,000 transgenic mice and screen them for one which has site-specific integration of foreign DNA. Thus, for the present, injection of DNA for the purpose of improving animal genotypes is likely to incur some loss secondary to insertional mutagenesis.

Variable expression of foreign genes is also a problem. In some instances the variability of expression is related to insertion site (42) or to the presence of plasmid or bacteriophage sequences linked to the injected gene, which may inhibit expression (29, 43, 44). In still other cases, however, unstable expression is poorly understood. In several interesting pedigrees studied by Palmiter et al (34), mice carrying and expressing a foreign gene frequently sired offspring which inherited the new gene but did not express it. Occasionally, progeny of such transgenic mice manifested increased expression relative to their parents. This finding was hypothesized to be due to the absence of “organizing sequences” on the injected DNA construct. The foreign sequence was the herpes-

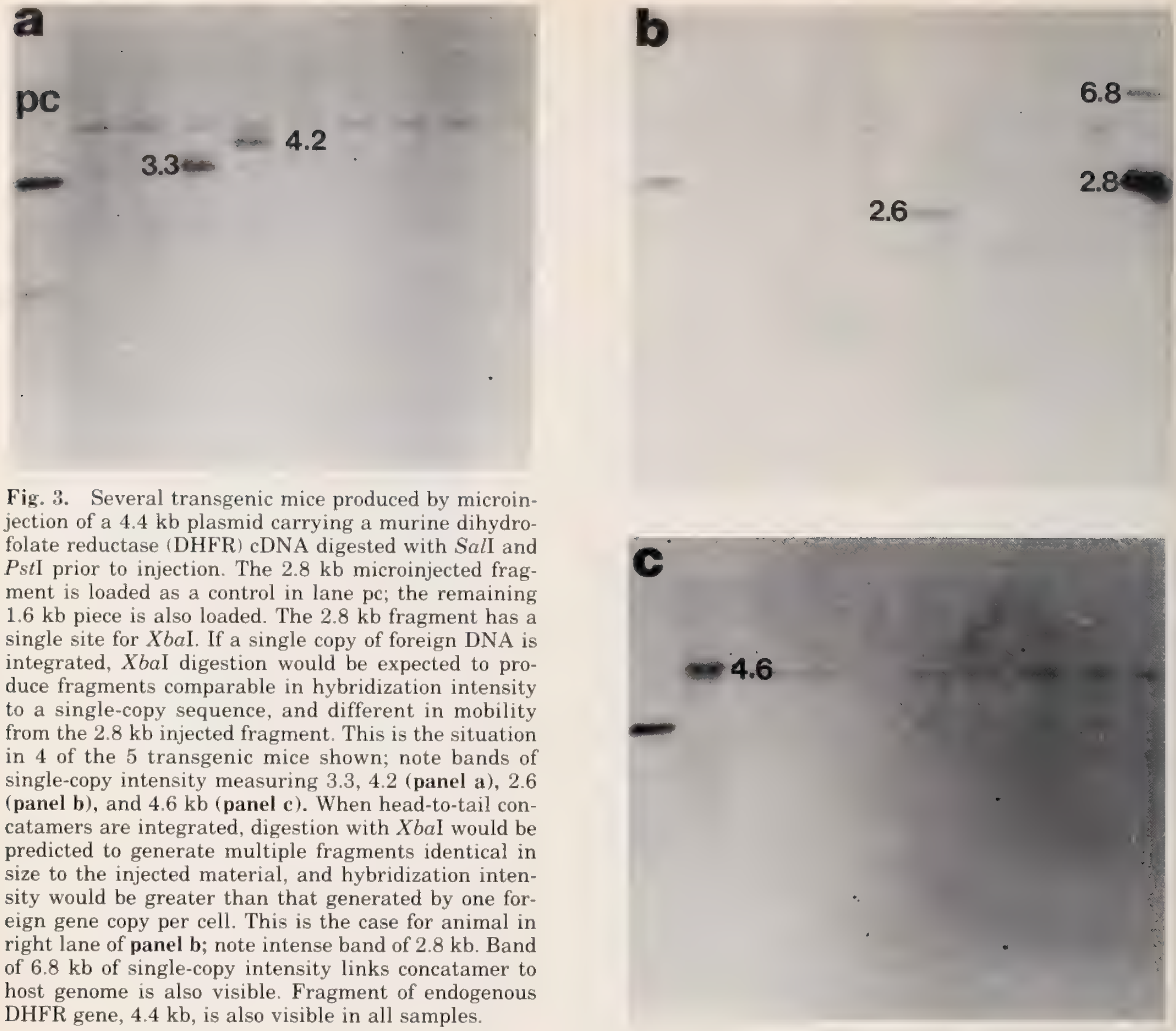

Fig. 3. Several transgenic mice produced by microinjection of a 4.4 kb plasmid carrying a murine dihydrofolate reductase (DHFR) cDNA digested with *Sal*I and *Pst*I prior to injection. The 2.8 kb microinjected fragment is loaded as a control in lane pc; the remaining 1.6 kb piece is also loaded. The 2.8 kb fragment has a single site for *Xba*I. If a single copy of foreign DNA is integrated, *Xba*I digestion would be expected to produce fragments comparable in hybridization intensity to a single-copy sequence, and different in mobility from the 2.8 kb injected fragment. This is the situation in 4 of the 5 transgenic mice shown; note bands of single-copy intensity measuring 3.3, 4.2 (**panel a**), 2.6 (**panel b**), and 4.6 kb (**panel c**). When head-to-tail concatamers are integrated, digestion with *Xba*I would be predicted to generate multiple fragments identical in size to the injected material, and hybridization intensity would be greater than that generated by one foreign gene copy per cell. This is the case for animal in right lane of **panel b**; note intense band of 2.8 kb. Band of 6.8 kb of single-copy intensity links concatamer to host genome is also visible. Fragment of endogenous DHFR gene, 4.4 kb, is also visible in all samples.

virus thymidine kinase gene, which was thought not to have organizing sequences present on mammalian genes whose role is to stabilize expression across generations (39). This hypothesis does not explain why the murine α-fetoprotein gene may also be variably expressed within transgenic mouse pedigrees (29). Therefore the problem of variable expression has not been entirely solved, though removal of sequences derived from cloning vectors has been shown to lead to increased expression (29, 43).

Another bothersome characteristic of some transgenic mice is that the structure of transferred DNA is unstable. Sequence instability falls into two categories.

First, homologous subunits of large head-to-tail concatamers in foreign DNA may recombine with each other and excise themselves. This phenomenon is observed as a loss of gene copy number in transgenic offspring relative to their parents. This kind of problem only occurs when more than one copy of the foreign sequence is integrated. However, because it can be associated with changes in expression (34), such instability can be troublesome.

Yet another type of instability, however, consists of changes in the structure of the integrated fragment. Restriction mapping of progeny in one lineage (45) has shown a clear change in restriction pattern in some progeny. In this case the lineage harbored a concatamer of the foreign sequence, so it was not clear that this change differed qualitatively from the internal recombinational sequence instability described earlier. However, data clearly show (Fig. 2) that even when one copy of foreign material is integrated in transgenic mice, it can undergo structural alteration over time. Why foreign DNA can occasionally be unstable in transgenic mice is unclear, but this instability may relate to integration

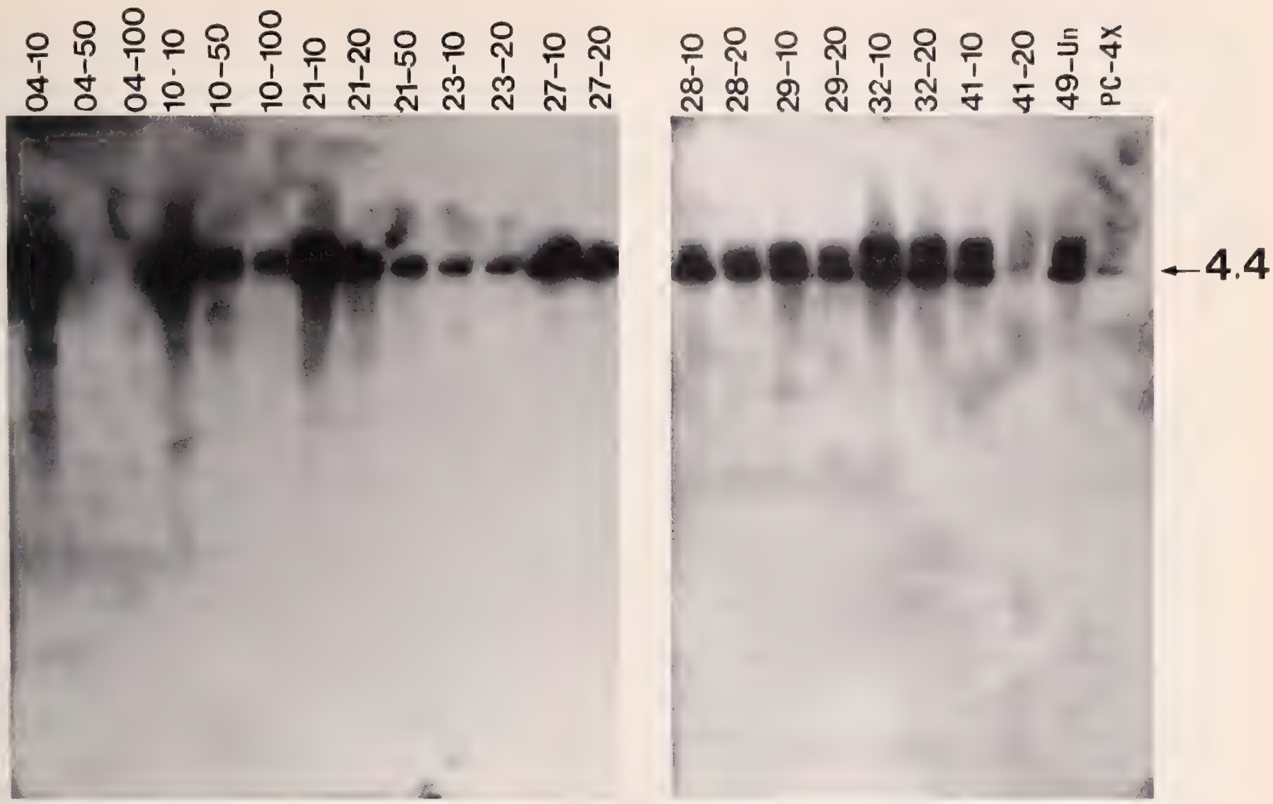

Fig. 4. Series of transgenic mice microinjected with same 4.4 kb DHFR plasmid, linearized with *Pst*I prior to injection. All animals carry concatamers of the foreign plasmid, as shown by intense 4.4 kb *Xba*I fragment in all samples. Length of concatamer has been estimated in this experiment by dilution of DNA and comparison of hybridization intensity to a 4 copy/cell control (right). Prefix is number of animal; suffix indicates degree of DNA dilution. Samples diluted 50 × and 100 × from mouse 04, and 20 × from mouse 41, were degraded secondary to overdigestion by restriction enzyme. Comparison of this figure with Fig. 3 shows that pretreatment of DNA with *Sal*I and *Pst*I leads preferentially to integration of single copies of foreign plasmid.

within chromosomal sites which are very sensitive to structural disturbances and are rendered unstable by exogenous DNA intergration.

Many of these problems could be solved if protocols could be developed to allow insertion of single, intact copies of foreign genes into the mouse genome. Not only would recombinational loss of gene copies be eliminated, but the regulation of the single foreign fragment would be far more easily studied. In the situation of single-copy integration, problems such as methylation could be evaluated in more detail (34) and changes in expression could be monitored closely. Our laboratory has produced some mice which suggest an approach to obtaining transgenic mice with single-copy insertions with high frequency. We have microinjected a recombinant plasmid, pFR400, which carries an altered dihydrofolate reductase gene cloned from cultured mouse fibroblasts (46). When the plasmid is digested with *Sal*I and *Pst*I prior to injection, it is integrated into transgenic mice as a single copy per cell with high frequency. Digestion of the same plasmid with *Pst*I alone, in contrast, gives concatameric integration in every case. These findings (Figs. 3, 4) suggest that digestion of foreign DNA with specific combinations of restriction enzymes can yield integration of one copy of foreign material with high frequency. We are currently exploring the possibility that double digestion with *Sal*I and *Pst*I will give single copy insertions of any foreign sequence.

Conclusions

Transgenic mice are exceedingly powerful tools for investigation of a variety of problems in mammalian gene regulation and are particularly useful in immunologic studies. The few animals already produced have provided important data on tissue specificity of immunoglobulin gene expression, regulation of gene rearrangement, and experimental alteration of the immune response. Functional dissection of the MHC region by insertion of subcloned gene fragments from this region into transgenic mice will certainly provide further insights into the regulation of this gene complex. The use of transgenic technology for genetic engineering of the immune response is already possible, but controlled experiments of this type await further improvements in this gene transfer system. Data are now appearing which suggest strategies for making such improvements. Directed integration of foreign genes into mammalian cells and controlled insertion of single copies of donor material into mice now seem possible.

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Letters to the Editor

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Gene Therapy: Creating Mosaic Pattern of Drug Susceptibility

To the editor: In the June 1985 *Mount Sinai Journal* report of the symposium on combination cancer chemotherapy, Bierman adumbrates an approaching reality of genetic intervention for individuals at high cancer risk by stating that "although limited in application now, gene therapy promises to become decisive in all of preventive and protective medicine" (1). Although he does not specify what type of genetic strategy might succeed, one possibility is suggested by the acknowledgment of several other contributors that current cancer therapy is often thwarted by its inability to eradicate tumors without killing an intolerably large fraction of normal cells. Since preservation of even a relatively small minority of normal stem cells can ensure host survival, one potential means of improving the prospects for successful chemotherapy in high-risk individuals who might later develop a malignancy entails the prophylactic insertion of genes determining sensitivity to chemotherapeutic drugs. The genes must be inserted into tissues in a scattered fashion, so that in any given tissue, some cells acquire a given gene while others do not, resulting in a *mosaic* pattern of differing drug susceptibilities. Since most human tumors are clonal—arising from a single cell—and since a clone arising in a mosaic host must inevitably differ from at least some of the other cells in the mosaic, a neoplasm that develops in a mosaic individual should be treatable with therapy aimed at its drug susceptibility characteristics, while host survival is protected by those normal cells in the mosaic that do not share those characteristics.

Tests of this strategy in experimental animals should become feasible soon through application of the virus-based gene vectors cited by Bierman. That these tests might succeed is suggested by recent experiments in my laboratory involving the gene for the thymidine kinase (TK) enzyme specified by herpes simplex virus (HSV). Guanosine analogs such as acyclovir are specific substrates for HSV-TK and selectively kill cells expressing the HSV-TK gene. When tumor cells bearing an inserted HSV-TK gene were implanted into mice, I found that the resulting tumors were almost universally curable with these analogs, whereas the tumors were always fatal when untreated and responded poorly to conventional chemotherapy.

Clearly, much more experimentation will be needed to determine the extent to which gene insertion can create potentially exploitable differences between cells in terms of drug sensitivity, but if tests in animals yield favorable results, application to high-risk human groups is not inconceivable in the foreseeable future. If genetic technology becomes safe and efficient enough in the more remote future, use of the strategy may even be possible in the general population.

1. Bierman HR. Multiple primary neoplasms and tumor diathesis. Mt Sinai J Med 1985: 52:435–442.

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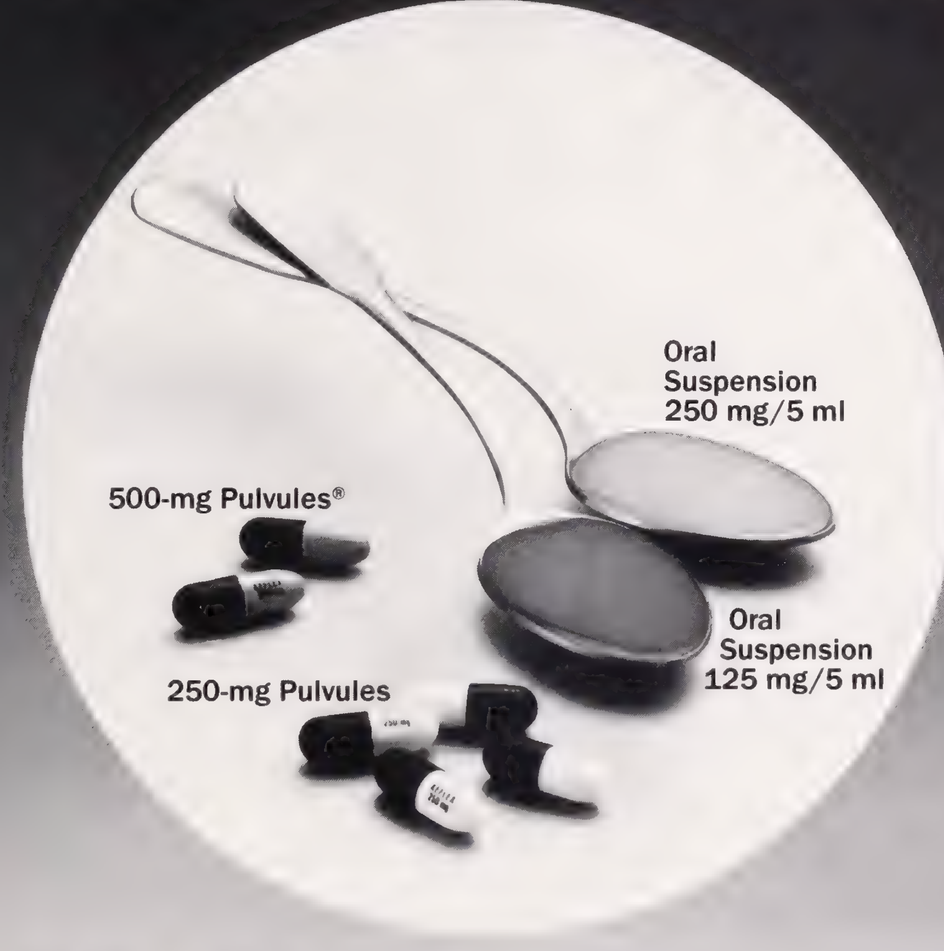
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THE MOUNT SINAI JOURNAL OF MEDICINE
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The Cervical Cap: Effectiveness, Safety, and Acceptability as a Barrier Contraceptive

NIELS H. LAUERSEN, M.D., KATHLEEN H. WILSON, B.S., ZOE R. GRAVES, PH.D., FORD CALHOUN, PH.D., LORI E. LEEDS, B.S., AND YANNI ANTONOPOULOS, PH.D.

Abstract

The experiences of 217 volunteers fitted with the cervical cap—a contraceptive barrier device used widely in Europe, currently under consideration by the Food and Drug Administration for release in the United States—were analyzed to assess the cap's effectiveness. In 24 months of use, 43 pregnancies were reported among 39 women (4 became pregnant twice on the cap). The Pearl Index was 22.7 pregnancies per 100 woman-years of use. Neither the characteristics of participants nor the characteristics of cap use affected the pregnancy rate significantly. However, the pregnancy rate was higher in participants reporting an average frequency of intercourse of 20 or more times per month and in those who observed cap displacement sometime during intercourse. Medical problems associated with cap use were minimal. Increased vaginal odor was the side effect reported most frequently. The majority of participants deemed the cap a positive contraceptive; 83% stated they would recommend it to friends.

A growing concern, stimulated by the popular media, over the actual and perceived risks associated with oral contraceptives and the intrauterine contraceptive device has led many women to return to more conventional methods of barrier contraception. The search for alternative contraception has led to revival of interest in the cervical cap, a contraceptive device widely used in Europe since the nineteenth century. American interest in the cervical cap came much later and can be traced to the 1970s; it was largely spurred by various women's health groups.

The cervical cap is a barrier method similar to the diaphragm which places a physical barrier between the sperm and the uterus by occluding the cervical canal. Attractive features of the cervical cap are its small size, its comfort and convenience, and the fact that it can be worn for a longer period of time than the diaphragm.

In 1953, Tietze and co-workers (1) studied 143 women and found the cap to be 98% effective among the 101 women who continued to use it. Subsequent studies, however, challenged the efficacy and safety of the cap (2, 3). Zodhiates and associates (2) reported frequent unwanted pregnancies, cap displacement during intercourse, and discomfort to the sexual partner.

Further information on the contraceptive effectiveness, convenience, and possible side effects of the cervical cap is required to provide a rational basis for the evaluation and the improvement of the cap as an alternative method of birth control.

Participants and Methods

The Cervical Cap. The present study examines the contraceptive effectiveness and side effects of the Prentif Cavity-Rim Cervical Cap (Lamberts (Dalston) Ltd., 202 Queensbridge Road, London,

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England). This thimble-shaped rubber dome (Fig.) is available in four sizes:

Inside Diameter	Depth
22 mm	34 mm
25 mm	36 mm
28 mm	38 mm
31 mm	40 mm.

All four sizes were utilized in this study. The cap fits directly over the cervix and acts as a mechanical barrier to prevent the passage of sperm through the cervical canal into the uterus. The cap should fit closely over the cervix and is held in place both by the cap's circular rim, which embeds in the vaginal muscle tissue, and by suction.

Participants. All women who were fitted with cervical caps were volunteers. These women had been referred for inclusion in this investigation by women's health groups, physicians, and other cap users. In addition, some women sought inclusion after learning about the cap through the media. The status of the cap as an investigational device was explained to all candidates and informed consents were obtained prior to examination and fitting.

Fitting. All candidates were examined and fitted for the cervical cap by the principal investigator. Initially, a detailed medical history was obtained, focusing on previous contraception and fertility; this was followed by a complete physical examination. If the pelvic examination revealed uterine fibroids, endometriosis, cervical erosion, or other anatomical abnormalities, the volunteer was eliminated as a candidate for the cervical cap. In each of the remaining candidates, a Pap smear was obtained for cytologic evaluation and an attempt was made to fit the cap. The principal investigator assessed cervical size through both manual and visual inspection and, in general, a woman was initially fitted with the smallest possible cap, progressing to larger caps, to ensure proper suction and snug fit. A proper fit is characterized by a good seal and adequate suction.

Instruction of Participants. Each received instruction in the contraceptive mechanism of the cervical cap. After the proper size cap was determined, that woman was instructed on the proper method of insertion and removal of the cap. In addition, she was advised to fill the cap approximately one-quarter full with spermicidal jelly prior to insertion and to leave the cap in place for at least six hours, but not more than 48 hours, after intercourse. Each participant was required to insert the cap by herself at least three times at the time of the fitting, and its proper placement was checked by the principal investigator. The participant was also required to remove the cap by herself to demonstrate her facility with this aspect of the procedure. Participants were advised to continue practicing insertion and removal at home. Furthermore, it was suggested that each woman use a backup form of contraception for the first two weeks during intercourse to provide added protection until she was secure with this contraceptive technique. If, at any time, she detected that the cap had become dislodged during intercourse, she was advised to return to have the cap refitted and to use alternate contraception until the refitting.

Fig. The rubber Prentif cervical cap.

The instructions for care of the cap were similar to those for the diaphragm and included careful washing in soap and water and dusting with cornstarch until reuse. Participants were advised that the fit of the cap should be checked by a physician routinely every six months, and in addition after certain events, such as pregnancy or abortion. In addition, the cap was to be inspected regularly and replaced if it showed any sign of damage or degeneration of the material. The women were also advised to obtain a Pap smear annually.

Study Design. Approximately 600 women were contacted and invited to participate in this retrospective study; 217 women who had been fitted with the cap for 4 to 36 months agreed to participate. Each of them filled out an anonymous and comprehensive questionnaire designed to determine efficacy and patient perception of the cervical cap as a contraceptive device. Solicitation of information via a mailed questionnaire was employed primarily because participants were geographically dispersed, making in-person follow-up visits to most of them impractical. Furthermore, the need to preserve their anonymity, in the light of the sensitive nature of the information sought, precluded the use of telephone interviews.

Results

Characteristics of Study Population. The 217 participants tended to be well educated; 75% had finished college and 22% had advanced degrees. The respondents were in their mature, fertile years; 84% were under 35 years of age; the mean age was 29 (range 21 through 43 years). These women were sexually active, frequency of intercourse averaging 13 times per month. Of these women, 48% had never been pregnant and approximately the same percentage, 48.4%, had had at least one abortion; 26 women (12.0%) had living children.

Characteristics of Cervical Cap Use. The cervical cap can be inserted and left in place for a longer period of time than can the conventional diaphragm. Approximately three-fourths of the women in this study routinely wore the cap for one to two days, and 17% routinely wore it for three or more days; 44% left the cap in place for three or more days during some period of use. In general, participants followed instructions and left the cap in place for at least six hours after intercourse; half removed the cap at between six and eleven hours after intercourse. An average of 88% used a spermicide in conjunction with the cap. In addition, 31% alternated the cap with another form of contraception. The alternate form of contraception was either a diaphragm, condom, or foam, which was used while the woman was menstruating.

Contraceptive Efficacy. Two hundred five women reported they had used the cap for a total of 2,274 months; the average was 11.1 months. There were 43 cap pregnancies in 39 women in the study population. By the Pearl formula, this yields 22.7 pregnancies per 100 woman-years of use. The characteristics of those in the study population who became pregnant with the cap ("cap-pregnant") were compared statistically to those who did not become pregnant ("not-cap-pregnant"). The 39 cap-pregnant women tended to be slightly younger (mean age, 27.5 years), slightly less likely to have graduate degrees (Table 1), and more likely to have had previous pregnancies and previous abortions (Table 2)—but none of these differences approached statistical significance.

TABLE 1
Comparison of Age and Education of Cap-Pregnant vs. Not-Cap-Pregnant Study Population

	Cap pregnant	Not cap pregnant
	No. (%)	No. (%)
Age (yrs)		
25	9 (23.1%)	28 (15.8%)
25–29	17 (43.6%)	68 (38.4%)
30–34	11 (28.2%)	48 (27.1%)
35–39	2 (5.1%)	26 (14.7%)
40+	0 (0%)	7 (4.0%)
Total responding	39	177
Education (yrs)		
12	1 (2.6%)	0 (0%)
12–15	7 (17.9%)	46 (26.1%)
16–17	26 (66.7%)	88 (50.0%)
18+	5 (12.8%)	42 (23.9%)
Total responding	39	176

The average frequency of intercourse per month was higher (16 times per month) for cap-pregnant than for not-cap-pregnant women (12 times per month). This difference was not statistically significant ($t = 1.48$, $p > 0.05$). However, 36% of women with average intercourse frequency of 20 or more times per month became cap-pregnant, whereas only 15% with a frequency of less than 20 times per month became cap-pregnant (Table 3). Statistical analysis of a 20-times-per-month breakdown showed a significant correlation of intercourse frequency and oc-

TABLE 2
Comparison of Obstetric History of Cap-Pregnant vs. Not-Cap-Pregnant Study Population

	Cap pregnant	Not cap pregnant
	No. (%)	No. (%)
Prior deliveries		
None	35 (89.7%)	163 (94.7%)
1	2 (5.1%)	13 (7.6%)
2	1 (2.6%)	4 (2.3%)
3	1 (2.6%)	1 (0.5%)
Total responding	39	172
Prior abortions		
None	16 (41.0%)	93 (54.1%)
1	15 (38.5%)	45 (26.2%)
2	5 (12.8%)	14 (8.1%)
3	2 (5.1%)	11 (6.4%)
4	0 (0%)	7 (4.1%)
5	0 (0%)	2 (1.2%)
6	1 (2.6%)	0 (0%)
Total responding	39	172

TABLE 3

Frequency of Intercourse of Cap-Pregnant vs. Not-Cap-Pregnant Study Population

	Cap pregnant	Not cap pregnant
	No. (%)	No. (%)
Intercourse frequency (average per mo.)		
5	0 (0%)	21 (13.4%)
5–9	10 (26.3%)	41 (26.1%)
10–14	7 (18.4%)	44 (28.0%)
15–19	6 (15.8%)	24 (15.3%)
20–24	8 (21.0%)	12 (7.6%)
25–29	2 (5.3%)	8 (5.1%)
30+	5 (13.2%)	7 (4.4%)
Total responding	38	157

currence of cap pregnancy. It appears it was not simply the difference in frequency of intercourse that affected the pregnancy rate, but frequency of intercourse in conjunction with the use of the cervical cap. Of the 42 women who stated an intercourse frequency of 20 or more times per month, 23 (53%) had abortions prior to cap use; of the 153 women who stated an intercourse frequency of less than 20 times per month, 73 (47.7%) had prior abortions. This difference was not statistically significant ($p > 0.05$, $\chi^2 = 0.40$).

Neither length of time, in toto, that a woman wore the cap nor the period of time a woman left the cap in place after intercourse appeared to influence the pregnancy rate. The use of spermicide also appeared to have minimal effect; the pregnancy rate was 19% (34/179) among women who used a spermicide with the cap and 20% (5/25) among those who used no spermicide. Neither did the use of alternate contraception significantly alter the pregnancy rate. Of 64 women using alternate contraception, 10 became pregnant, whereas 29 of 141 using the cap alone became pregnant; this difference was not statistically significant ($\chi^2 = 0.42$, $p > 0.05$).

A statistically significant correlation was established between reported dislodgement of the cap at some time during intercourse and cap pregnancy. Of the 39 respondents who became pregnant, 27 (71%) reported that the cap became dislodged. In contrast, only 44% of the not-cap-pregnant women reported that the cap had never become dislodged during pregnancy; this difference was statistically significant ($p < 0.01$). Several cap-pregnant respondents themselves attributed their pregnancies to dislodgement of the cap. Of the 39 cap-pregnant women, 22 attributed pregnancy exclusively to cap failure, 10 attributed it to both contraceptive and human failure, and 7 women responded that cap pregnancy was due exclusively to human error. The specific month of cap use in which a participant became pregnant can be determined from the questionnaires. Of 27 pregnancies reported, 24 (89%) occurred during the first year of cap use; however, one woman became pregnant after 18 months of use (Table 4).

Continuation Rate. Of those who responded to the questionnaire, 136 women (62.7%) indicated continuing use of the cap; 81 women had discontinued use—19.7% during the first month and 64.5% prior to the sixth month of use (Table 5). Unplanned pregnancy was the primary reason for discontinuing, although 14 of the 39 cap-pregnant women continued to use the cap. Responses from 12 women indicated they had experienced such difficulty with insertion or removal, or both, that they discontinued use; discontinuation for this reason occurred prior to six months of use. Responses from 5 women indicated they had discontinued use of the cap for medical reasons, including vaginal infection (2), cervicitis (1), pelvic inflammatory disease (1), and physician's advice (1). However, 15 women, 18.5% of those who discontinued use, did so for reasons unrelated to the effectiveness or effects of the device itself: 9 because they had discontinued sexual relations, 5 because they wished to become pregnant, and one because she became menopausal (Table 6).

Side Effects. Respondents were extensively queried on their perceptions of the comfort and convenience of cap use and any potential medical problems associated with it. Approximately three-fourths of the respondents reported no diffi-

TABLE 4

Time of Cap Pregnancy

(n = 27)

Month of use pt. became pregnant	Patients No. (%)	Cumulative rate (%)
<1	1 (3.7)	1 (3.7)
1	3 (11.1)	4 (14.8)
2	3 (11.1)	7 (25.9)
3	4 (14.8)	11 (40.7)
4	2 (7.4)	13 (48.1)
5	2 (7.4)	15 (55.6)
6	1 (3.7)	16 (59.2)
7	2 (7.4)	18 (66.7)
8	1 (3.7)	19 (70.4)
9	3 (11.1)	22 (81.5)
10	1 (3.7)	23 (85.2)
11	1 (3.7)	24 (88.9)
12	1 (3.7)	25 (92.6)
13	1 (3.7)	26 (96.3)
18	1 (3.7)	27 (100%)

TABLE 5
Rate of Continuation of Use of Cervical Cap, Maximum and Minimum Periods

Period of Use	Users No. (%)	Discontinuers No. (%)
Maximum		
<1 month	15 (7.3%)	15 (19.7%)
1–5 months	40 (19.5%)	34 (44.7%)
6–11 months	49 (23.9%)	19 (25.0%)
12–17 months	66 (32.2%)	7 (9.2%)
18+ months	35 (17.1%)	1 (1.3%)
Total responding	205	76
Minimum		
<1 month	205 (100%)	0 (0%)
1 month	190 (92.7%)	15 (19.7%)
6 months	150 (73.2%)	49 (64.5%)
12 months	101 (49.3%)	68 (89.5%)
18 months	35 (17.1%)	75 (98.7%)
>18 months	34 (16.6%)	76 (100%)
Total responding	205	76

culty with insertion and no discomfort to themselves. Partner discomfort, however, was somewhat higher; 78 of 203 respondents stated that their sexual partners complained of discomfort caused by the cap during intercourse. The primary complaint about the cap itself was the problem of malodor, as reported by 53 of 197 respondents (27%). Medical side effects of cervical cap use were few. An increase in the incidence of cervical laceration or ulceration was reported by 4 participants; 2 of these experienced increased cervicitis as well, and another 2 respondents observed increased cervicitis only. An increase in vaginal odor was the most frequently reported medical side effect of cap use (Table 7).

Of the 136 women who, at the time of their response to the questionnaire, reported they were continuing their use of the cervical cap, 69 (50.7%) reported neither discomfort nor any medical problem with continued use. In addition, 83% of the respondents stated they would recommend the cap to their friends as a contraceptive device.

Discussion

The data provided by the 217 cervical cap users in our study indicate the major problem with the cap as a contraceptive technique is its effectiveness. Of 217 respondents, 39 (18%) became pregnant while using the cap, and four of these women became pregnant twice while on the cap. The pregnancy rate among this group, however, appears similar to their previous experiences with the diaphragm. Of the 196 women who reported they had employed a diaphragm as a previous form of contraception, 29 (14.8%) stated they had experienced unplanned pregnancy/pregnancies while on the diaphragm. Furthermore, these results are similar to those reported for the diaphragm (4)—a range of effectiveness from 2.2 to 28.9 pregnancies per 100 woman-years of use.

An examination of the data provided by women who became cap-pregnant may help to minimize the pregnancy rate for future cervical cap users. A definite correlation existed in our study between reported dislodgement of the cervical cap during intercourse and cap pregnancy. This displacement may have been due to improper fit of the cap, or to improper insertion of the cap by the user, or to both. Only four sizes of the cap are currently available; this choice apparently fails to supply the ideal fit for a number

TABLE 6
Timing and Reasons for Discontinuing Cervical Cap

Reasons for Discontinuing	No. pts. (%)	Time discontinued <1 mo	<1 yr	1 yr+	No answer
Unplanned pregnancy	25 (30.8%)	1	20	3	1
Difficulty with insertion/removal	12 (14.8%)	6	6	—	—
No sexual relations	9 (11.1%)	3	6	—	—
Partner's discomfort	8 (9.9%)	1	7	—	—
Cap dislodged during intercourse	6 (7.4%)	1	3	1	1
Desire to become pregnant	5 (6.2%)	—	5	—	—
Medical reasons	5 (6.2%)	1	2	—	2
Cap inconvenient	4 (5.0%)	1	2	1	—
Lack of confidence	3 (3.7%)	—	2	—	1
Partner's vasectomy	2 (2.5%)	—	—	2	—
Menopause	1 (1.2%)	—	—	1	—
No reason given	1 (1.2%)	1	—	—	—

TABLE 7
Problems with Cervical Cap Use

Problem	No. yes (%)	No. no (%)	No answer
Difficult to insert	57 (27.0%)	154 (73.0%)	6
Wearer discomfort			
Insertion removal	31 (16.1%)	161 (83.8%)	25
Intercourse	42 (22.1%)	148 (77.9%)	27
Daily wear	38 (20.3%)	149 (79.7%)	30
Partner discomfort	78 (38.4%)	125 (61.5)	14
Malodor of cap	53 (26.9%)	144 (73.1%)	20
Increased incidence:			
Cervical laceration or ulceration	4 (2.1%)	187 (97.9%)	26
Cervicitis	4 (2.1%)	186 (97.9%)	27
Fungal infection	9 (4.7%)	181 (95.3%)	27
Cystitis	12 (6.3%)	179 (93.7%)	26
Vaginal infection	26 (13.5%)	166 (86.4%)	25
Vaginal discharge (not infection)	22 (11.5%)	169 (88.5%)	26
Vaginal odor	29 (15.2%)	161 (84.5%)	27

of women. If the cap is slightly too large, suction will be less than required and dislodgement is quite possible. If the cap is slightly too small, it might be so difficult to insert or remove that the woman will not use it routinely because of the inconvenience. A broader range of cap sizes could alleviate this aspect of the problem.

The potential release by the Food and Drug Administration of the cervical cap for routine use intensifies the need for current information on this contraceptive technique. The study we report here appears to indicate that participant acceptance rose and cap failure dropped during the second year of use. Furthermore, the cap compares favorably with the diaphragm. The pressing need for an increased range of choice in contraceptive techniques dictates that investigations of the cervical cap be continued in the hope that it soon can be made generally available.

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A New Overpressure Safety Valve for Use in the Venting Line during Cardiopulmonary Bypass

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Abstract

A new overpressure safety valve has been developed for use in the left ventricular venting line during cardiopulmonary bypass. It consists of three valves assembled in a single unit that prevents the introduction of air into the left ventricle that may occur during venting of the left ventricle.

Venting of the left ventricle during cardiopulmonary bypass, although still a controversial issue, is commonly used in most cardiac interventions requiring extracorporeal circulation. Decompression of the left ventricle is usually accomplished through a catheter introduced in the right superior pulmonary vein or through an aortic root vent. A major risk of direct left ventricular venting, however, is the introduction of air and its subsequent embolization into the systemic circulation. This may be caused by excessive negative pressure applied by the roller pump to the venting line or by incorrect placement of the pump tubing with reversal flow.

To overcome these problems a new overpressure safety valve has been designed.

Technique

The new William Harvey H-130 overpressure safety valve consists of three different valves assembled in a single unit (Figs. 1, 2). Valve 1 is a fishmouth valve that allows blood to drain in only one direction, from the patient toward the cardiotomy reservoir. Valve 2 prevents the fishmouth valve from inverting by venting positive pressure to atmosphere in case of flow in the wrong direction. Valve 3 is a negative-pressure safety valve designed to avoid excessive negative pressure from building inside the left ventricle since it activates at approximately −190 mmHg at 0.5 liter/min flow. This disposable plastic valve is placed between the venting catheter and the cardiotomy reservoir and is now available for clinical use. (Bard Cardiosurgery Division, C. R. Bard, Inc., P.O. Box M, Billerica, Massachusetts 01821).

Discussion

Although the need for left ventricular venting during cardiopulmonary bypass is controversial, there are several advantages associated with its use.

First, it empties the blood that returns to the left ventricle through the bronchial circulation and thus prevents distention of the left ventricular chamber. This is particularly important in the presence of aortic insufficiency (1). There is conclusive evidence in the literature that left ventricular distention reduces subendocardial perfusion, causing persistent ischemia, and results in impaired ventricular performance (2).

Second, venting of the left ventricle facilitates exposure for distal coronary anastomosis and reduces the rate of myocardial rewarming by

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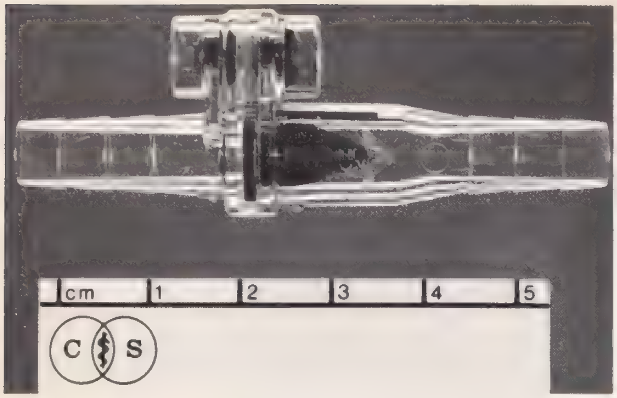

Fig. 1. Top Newly designed overpressure safety valve.

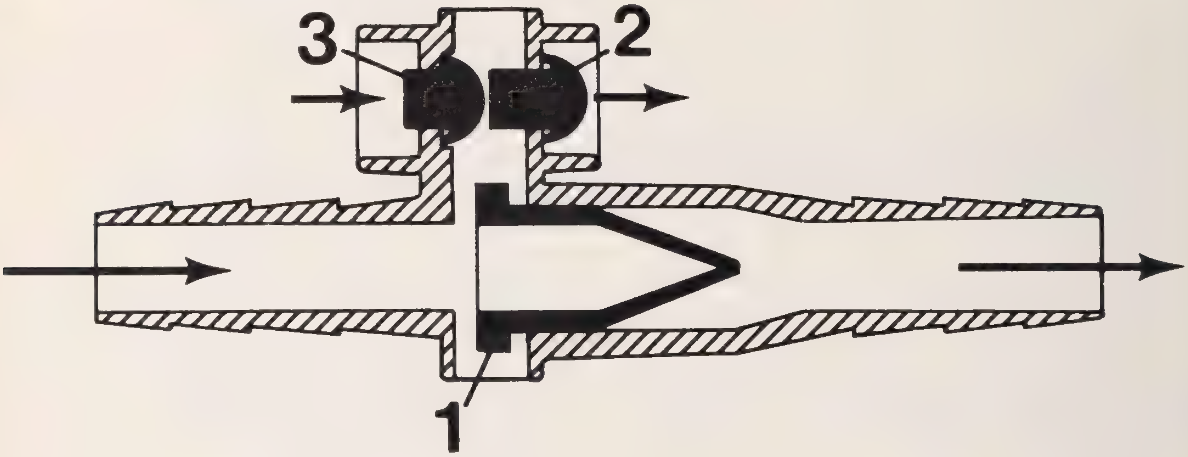

Fig. 2. Bottom Diagram showing (1) fishmouth valve, (2) positive-pressure safety valve, and (3) negative-pressure safety valve. Arrows indicate direction of flow.

effectively draining the blood returning to the left ventricle. It also prevents blood going into the coronary circulation washing out cardioplegic solution.

However, venting is not without risks, and complications may arise, particularly the introduction of air into the left ventricle with subsequent embolization into the systemic circulation. In addition, it creates a potential source of bleeding and may produce myocardial damage, dislodgement of endocardial thrombus, arrhythmias, and pulmonary venous occlusion (3).

Despite these disadvantages we believe that left ventricular venting is not only advisable but essential in most operations requiring extracorporeal circulation. Based on our experience in more than 200 consecutive cases with the new overpressure safety valve, we believe it effectively prevents the accidental introduction of air into the systemic circulation that may occur with the use of a left ventricular venting line during cardiopulmonary bypass. However, we cannot overemphasize the need to vent the left ventricular apex with an aspirating needle to assure complete evacuation of air from the left ventricle following removal of the vent line.

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Effect of Diet on Dissolution of Gallstones by Ursodeoxycholic Acid, including a Comparison between Ultrasonography and Cholecystography

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Abstract

The aim of this study was twofold: to make a determination in a randomized, prospective trial, first, the effect of different diets on ursodeoxycholic acid (UDCA)-induced desaturation of biliary cholesterol and dissolution of gallstones and second, the reproducibility of and agreement between oral cholecystography and ultrasonography in assessing dissolution. Sixty-nine patients with radiolucent gallstones were randomized to one of four diets while receiving UDCA (750 mg hs) for 21 months. The four diets were: standard (500 mg cholesterol/day), low cholesterol (250 mg/day), added bran (30 g/day), and substituted medium-chain triglycerides (MCT) oil (20% of fat). Dietary intake and good compliance were verified by computerized analysis of dietary diaries. Masked two-way, side-by-side comparisons of the initial and final ultrasonogram and of the initial and final oral cholecystogram were done twice. No difference in the degree of desaturation or the frequency of dissolution of gallstones by UDCA occurred among the dietary groups. When gallstones were present by cholecystography, they invariably were detected by ultrasonography; however, ultrasonography detected stones not visualized by cholecystography, suggesting that the latter may have overestimated the frequency of dissolution (19% of patients by cholecystography versus 9% by ultrasonography).

Ursodeoxycholic acid (UDCA) desaturates bile and promotes the dissolution of cholesterol gallstones (1–8) by decreasing the hepatic secretion of biliary cholesterol (9). Since the rate of dissolution may be influenced by the degree to which bile is desaturated, dissolution by UDCA may be enhanced by diets that act to further decrease the saturation of cholesterol (10, 11).

Although the data are limited and conflicting, evidence exists to support the testing of a diet low in cholesterol to facilitate the dissolution of gallstones (12–14). Augmented dietary cholesterol for brief periods increased the biliary saturation of cholesterol in subjects with and without gallstones (15, 16). A diet low in cholesterol enhanced the effect of chenodeoxycholic acid (CDCA) on biliary saturation in patients with gallstones (12) and, with administration of CDCA at bedtime (13), reduced by approximately one-half the minimum effective dose necessary to desaturate bile. In addition, the rate of partial dissolution of gallstones after six months was more than twice that of the same dose of CDCA in three divided doses without dietary restriction. In

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the latter study, however, the conclusion could not be made that complete dissolution occurred more frequently or more rapidly (14). Furthermore, the effects of a bedtime dose and of a low-cholesterol diet were not separated.

The effect of substituting dietary medium-chain triglycerides (MCT) for long-chain triglycerides on hepatobiliary metabolism has been studied in the rhesus monkey (17). MCT decreased synthesis, pool, size, and secretion of bile acids in bile. Bile became less saturated, however, because the biliary secretion of cholesterol was decreased whereas the frequency of recycling of the pool of bile salts was increased. Diets supplemented with bran or other types of dietary fiber have been reported to increase the synthesis and pool of biliary CDCA and decrease saturation of cholesterol in patients with gallstones (18–19). No controlled trials have been conducted, however, assessing MCT or bran as adjuvant therapy in the medical dissolution of gallstones.

The advent of gallbladder ultrasonography (US) has provided a rapid, accurate, noninvasive, and nonionizing alternative to oral cholecystography (OCG) for detection of gallstones (20–26). In previous studies, successful dissolution of gallstones has been defined as diminution in size or disappearance of gallstones assessed usually by OCG alone (27). Although a suggestion has been made that US is more sensitive than OCG, no prospective, masked studies comparing gallbladder ultrasonography and oral cholecystography in this assessment have been reported.

The aims of the current study, accordingly, were to study (a) the effect of diet (low cholesterol, bran supplemented, or MCT substituted) on the efficacy and safety of UDCA-induced dissolution of gallstones and (b) the reproducibility of and agreement between OCG and US in assessing dissolution.

Methods

Patients. Patients included in the study weighed less than 150% of ideal body weight. Women were postmenopausal, had had a hysterectomy or tubal ligation, or used an intrauterine device for contraception. The exclusion criteria were biliary colic or acute cholecystitis within one month of randomization; any prior jaundice due to extrahepatic obstruction of the bile duct, cholangitis, pancreatitis, or biliary tract surgery; acute viral hepatitis or drug-induced jaundice within the previous year; inflammatory bowel disease, active peptic ulcer, hyperlipidemia, anemia or leukopenia, impaired renal function, uncontrolled cardiac disease, or sensitivity to iodide. The results of laboratory tests at the time of evaluation that led to exclusion were serum aminotransferase values greater than twice the upper limit of normal, conjugated hyperbilirubinemia, fasting serum cholesterol greater than the upper limit of normal for age, fasting serum triglyceride greater than 500 mg/dl, or serum creatinine greater than 2.0 mg/dl. Other criteria that excluded participation were ingestion of more than 50 mg/day of bile acids for longer than three months, alcohol intake greater than 80 g/day, antihyperlipidemic drugs, anticholinergic drugs, estrogens in greater than replacement doses, and use of birth control pills.

Experimental Design. Sixty-nine patients with radiolucent gallstones were randomized to one of four diets for 21 months. All patients also received UDCA (750 mg hs) during this time. The diets were: standard (500 mg cholesterol/day); low cholesterol (250 mg/day); bran (30 g/day added to standard diet); or MCT oil substitution (average 17.3 ml/day to replace 20% of the fat calories/day). UDCA (Gipharmex, Milano, Italy) was 98% UDCA, 1.5% CDCA, and 0.5% other bile acids by gas-liquid chromatography. MCT oil (PVO International Inc., New York, New York) by weight was C8—69.8%, C10—27.2%, C6—3.0%, free fatty acid—0.3%, water—0.04%, and free glycerol—0.1%. Bran (Kansas State University, Department of Grain Sciences, Manhattan, Kansas) was produced from Hard Red Winter wheat. The bran particle size was 1–2 mm, and it was 9.5% crude fiber, 39.5% neutral detergent fiber, 5.0% ash, 11.3% acid detergent fiber, 3.4% lignin, 6.7% cellulose, and 0.3% pectin (Keltner Laboratories, Inc., Manhattan, Kansas).

Patients were seen every three months to determine adherence to diet, symptoms (including diarrhea, biliary pain, and dyspepsia), and weight and to obtain blood tests: bilirubin, SGOT, SGPT, alkaline phosphatase, GGT, serum total cholesterol (normal 135–315 mg/dl), LDL cholesterol (normal 62–185 mg/dl), HDL cholesterol (normal 29–77 mg/dl), and triglycerides (normal 30–135 mg/dl). During therapy, serum laboratory values were considered abnormally elevated if they were 10% or more above the upper limit of normal. Elevations of less than three times the upper limit of normal were considered minor elevations.

Prior to the study, dietary histories were obtained by a registered dietitian, and energy requirements were estimated using the Mayo Clinic Food Nomogram (*Mayo Clinic Diet*

Manual, W.B. Saunders, 1971). All participants were given individualized instructions for a weight-maintaining, solid-food, low-fiber (less than 5.0 g crude fiber per day) diet based on the Exchange Lists for Meal Planning (American Dietetic Association, 1976). The standard diet was calculated to provide 18%–22% of calories as protein, 38%–42% as carbohydrate, 38%–42% as fat (saturated and unsaturated), and 500 mg cholesterol/day. Alcohol, when requested, was limited to a maximum of 48 g/day, and instructions were provided for making appropriate substitutions for bread, fruit, and/or fat exchanges. All participants received a small diet scale for monitoring portion sizes.

Modifications of the standard diet were made for the specific dietary groups. For the low-cholesterol group, the standard diet was modified in protein sources and egg yolks to limit cholesterol to a maximum of 250 mg/day. For the bran group, the standard diet was supplemented with 30 g (9 tbsp) of unprocessed bran per day in divided doses. Participants were provided with the bran and instructions for incorporating it into the diet in an uncooked form. For the MCT oil group, 20% of the fat calories were replaced by MCT oil (114 kcal/15 cc). Participants were provided with the MCT oil and instructions for incorporating it into the diet.

Every three months during the study, the participants completed detailed food records for two specified periods of three consecutive days six weeks apart. Statistical analysis confirmed the validity of combining these three-day records to report mean daily intakes for the six days. Further analysis allowed combining the data obtained early (6–9 months) and late (18–21 months) in the study. The participants were seen for follow-up every three months to review food records and monitor compliance. Food records were submitted to the Nutrition Coding Center at the University of Minnesota for computerized nutrient analysis. All data were analyzed using the same edition of the nutrient data base.

OCG and US were done at 0, 9, and 21 months. Masked two-way comparisons of the initial and final OCG and the initial and final US were done twice. Each OCG was assessed for visualization of the gallbladder and the presence of gallstones. Gallstone characteristics, including configuration, floatability, number (1, 2, 3, 4–9, or greater than 10), calcification, and largest diameter (1–5, 5–10, 10–15, or greater than 15 mm), were recorded. Initial and final volumes of gallstones on OCG were calculated using the formulas of Baily et al (27) and were used to classify patients as having partial dissolution (greater than 50%) or nondissolution. Complete dissolution, when observed, was confirmed three months later by a repeat OCG and US, the patient having continued his assigned therapy. US examinations were assessed for the presence of gallstones, number (1, 2, 3, 4–9, or greater than 10), largest diameter (1–5, 5–10, 10–15, or greater than 15 mm), and acoustic shadowing.

The study was approved by the Cedars-Sinai Medical Center Human Subjects Committee and conducted according to the Principles of the Declaration of Helsinki. All patients signed informed consents.

Oral Cholecystography. Patients underwent cholecystograms in the fasting state after ingesting 3 g of iopanoic acid two consecutive evenings prior to the examination. A standard ballbearing for reference was taped to the patient's abdomen at the level of the gallbladder. Cholecystography was standardized by having uniform tube-to-film and patient-to-film distances. Fluoroscopic identification of the gallbladder was followed by overhead films that included supine, right posterior oblique, and right lateral decubital views. These were supplemented by upright views. Each examination delivered approximately 600 mrem to the skin and 60 mrem to the gallbladder.

Ultrasonography. Patients were examined in the fasting state using a B-mode Picker Echoview 80L or real-time Advanced Technology Laboratories scanner or both. A transducer frequency of 3.5 MHZ was utilized (13-mm medium-focus transducer). Scans were performed in the supine position using sagittal and transverse projections and in difficult evaluations included left lateral decubital views. Examinations routinely included scanning of the entire gallbladder, common bile duct, liver, and head of the pancreas. Cholelithiasis was diagnosed when strong intraluminal echoes and distal acoustic shadowing were present. Gallstone movement was assessed in equivocal cases.

Biliary Lipids. The determination of biliary lipid composition was done initially and at month 6 or 9 in all patients. Biliary cholesterol was determined by gas-liquid chromatography (GLC) (28). Bile acids were determined by the steroid dehydrogenase method for total bile acids (29) and by GLC for individual bile acids (28). Phospholipids were determined by colorimetry as digested phosphorus according to the method of Fiske and Subbarow (30). The saturation index (SI) was determined by the polynomial equation of Thomas and Hofmann (31) using the limits of cholesterol solubility of Hegardt and Dam (32). The SI also was

TABLE 1
*Pretreatment Characteristics of the 69 Randomized Patients**
(Mean ± SD)

Characteristic	Total	Standard	Low cholesterol	Bran	MCT
Number of patients	69	17	19	17	16
Age (years)	53.7 ± 10.9	52.4 ± 11.9	53.1 ± 9.6	54.4 ± 11.0	55.3 ± 11.7
Sex (number of women)	37	11	8	9	9
% Ideal wt	109.4 ± 17.9	110.0 ± 24.0	106.0 ± 15.2	111.5 ± 18.8	110.5 ± 12.4
Serum lipids (mg/dl)					
Total cholesterol	224.8 ± 39.1	242.2 ± 37.6	216.8 ± 34.9	223.9 ± 3.8	216.4 ± 43.2
LDL cholesterol	51.2 ± 14.5	55.1 ± 14.0	51.0 ± 11.1	44.9 ± 14.3	53.9 ± 17.5
HDL cholesterol	148.1 ± 36.1	160.0 ± 34.7	141.8 ± 36.4	150.1 ± 39.0	140.1 ± 33.6
Triglycerides	127.1 ± 72.5	137.3 ± 74.7	119.3 ± 41.0	144.2 ± 90.0	107.5 ± 79.2
Saturation index	1.62 ± .26	1.60 ± .27	1.65 ± .29	1.60 ± .25	1.63 ± .25
UDCA (% of bile acids)	1.6 ± 1.3	1.2 ± 1.1	1.8 ± 1.3	1.7 ± 1.5	1.6 ± 1.4
CDCA (% of bile acids)	30.8 ± 3.3	30.6 ± 3.9	30.8 ± 4.0	30.6 ± 3.1	31.5 ± 1.8
Gallstone volume (cm^3)	17.0 ± 34.9	16.1 ± 24.5	11.4 ± 16.5	28.7 ± 58.4	12.4 ± 25.5

* No significant differences were found among the dietary groups.

calculated using the Carey-Ko correction for UDCA-rich bile (9).

Statistical Methods. Methods of statistical analysis were based on Winer (33) and Cohen (34).

Diet. The unit of analysis was the mean daily intake of dietary components for each patient's dietary records. The mean intakes of the dietary components were compared among diets (standard, MCT oil, bran, low cholesterol), periods of study (6–9 months, 18–21 months), and between dietary records (the two three-day records) using repeated-measures analysis of variance models. Pairwise comparisons of diets were made using Tukey's multiple comparison procedure. For some comparisons, the homogeneity of variance assumption did not hold. Application of nonparametric methods (Mann-Whitney U-Test, Friedman's ANOVA, and Kruskal-Wallis ANOVA) in these cases, however, yielded similar results and led to the same conclusions as the parametric analyses.

OCG, US, and Biliary Lipids. Comparisons between dietary groups of the results of OCG, US, and analyses of biliary lipids were done using the chi-square test (noncontinuous variables) and the parametric one-way analysis of variance (ANOVA) or the Kruskel-Wallis nonparametric ANOVA (continuous variables). Reproducibility of US and OCG readings and agreement between the two methods were assessed using the Kappa statistic (35). Comparisons of results before and after therapy within diet groups were done using the paired t test.

All summary statistics are expressed as mean ± SD. The level of significance used was 0.05 after accounting for multiple analyses. All p values are two-sided.

Results

Patients. No significant differences were found in the pretreatment characteristics among the dietary groups (Table 1). All patients initially had saturated bile. The initial mean diameter of gallstones in the 37 patients who had single gallstones (16.0 ± 6.9 mm) was comparable among the dietary groups.

Dietary Analysis and Compliance. No statistically significant differences were found between diets for crude fiber and percent protein (Table 2). The mean intake of cholesterol was significantly ($p < 0.01$) lower in the low-cholesterol group than in each of the other groups. For percent carbohydrate, the MCT oil group had a significantly lower mean intake than the low-cholesterol group ($p < 0.05$). Correspondingly, for percent fat and total calories, the low-cholesterol group had a significantly lower mean intake than the MCT oil group (each $p < 0.01$).

In evaluating compliance to the specific dietary guidelines, all dietary groups were within the range for crude fiber (<5.0 g/day) and percent protein (18–22). The low-cholesterol group was above the range for percent carbohydrate and below for percent fat. The standard and bran groups were slightly below the range for percent fat. The low-cholesterol group complied with the <250-mg cholesterol limit. The mean cholesterol intakes for the other diet groups were each lower than the prescribed 500-mg level.

The bran group complied with a mean intake of 26.5 g/day of supplemental bran (88% of the prescribed amount). The MCT oil group complied with a mean intake of 17.3 ml/day of MCT oil (98% of the prescribed amount).

TABLE 2

Composition of Recorded Diets in the Four Dietary Groups in the 62 Patients Who Returned for Dietary Follow-up

Dietary component (mean ± SD)	Standard (n = 16)	Low cholesterol (n = 16)	Bran (n = 15)	Medium-chain triglycerides (MCT oil) (n = 15)
Cholesterol (mg)	404.2 ± 76.9	177.0 ± 58.2***	411.0 ± 106.3	439.1 ± 83
Crude fiber (g)	3.5 ± .9	3.7 ± 1.5	3.0 ± 1.6[a]	4.2 ± 1.50
% Protein	0.179 ± .026	0.184 ± .030	0.186 ± .041	0.180 ± .028
% CHO	0.421 ± .067	0.476 ± .085*	0.420 ± .059	0.393 ± .063
% Total fat	0.363 ± .057	0.316 ± .067**	0.372 ± .072	0.394 ± .049[b]
Total calories	1,687 ± 415	1,564 ± 407**	1,605 ± 432	1,910 ± 573

[a] Received in addition 30 g bran per day (2.9 g crude fiber per day).

[b] Includes substitution of MCT for 20% of total fat.

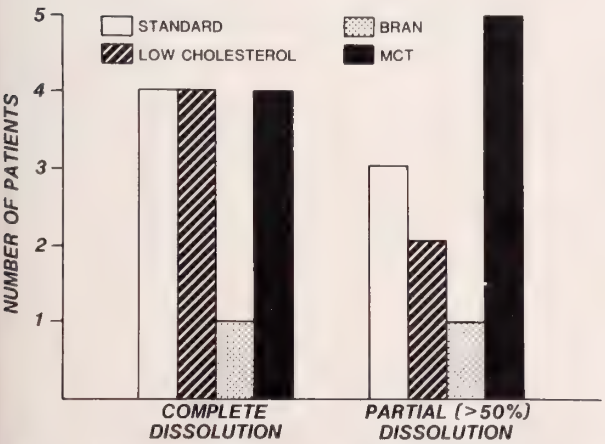
* $p < 0.05$ or ** $p < 0.01$ compared to MCT diet.

*** $p < 0.01$ compared to each of the other diets.

Dissolution. Figure 1 shows the number of patients with complete and partial dissolution by OCG at 9 or 21 months in each of the dietary groups. Thirteen (18.8%) of the 69 patients had complete dissolution and 11 others (15.9%) had partial dissolution. No significant difference in the number of patients achieving complete or partial dissolution was detected among the dietary groups.

No signficiant differences were found in percent of ideal body weight, serum cholesterol, or the presence of floating gallstones between patients with complete dissolution and patients with partial or nondissolution; however, patients with complete dissolution had a significantly smaller mean initial volume of gallstones than all other patients combined (5.9 ± 15.6 cm^3 versus 20.2 ± 38.2 cm^3, $p < 0.05$).

Treatment Failures. Forty-five (65.2%) of the 69 patients were classified as treatment failures (<50% dissolution, nonvisualizing OCG, and patients who did not complete the study) with no significant differences among the dietary groups (Fig. 2). Twenty-eight patients (40.6% of all patients) had less than a 50% decrease in the volume of gallstones and 3 others had a nonvisualizing gallbladder on the final OCG. Fourteen patients (20.3%) of all patients did not complete the study with no significant differences in the numbers or timing of losses among the dietary groups. The reasons for the loss of patients were as follows: 4 patients had acute cholecystitis requiring cholecystectomy; 5 patients had recurrent episodes of biliary colic and 4 of these patients had elective cholecystectomy; 3 patients lost interest in the study; and 2 patients died of



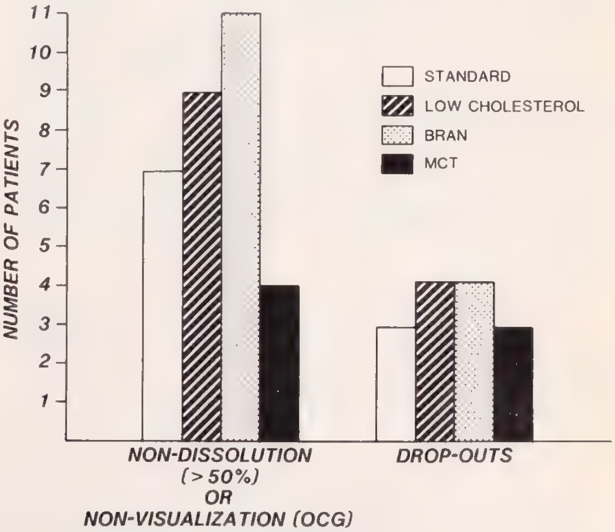


Fig. 1. **Left** Complete and partial dissolution among dietary groups (outcome on oral cholecystography). Frequency of dissolution was not significantly different among the groups.

Fig. 2. **Right** Treatment failures among dietary groups. Frequency of nondissolution or dropouts was not significantly different among the groups.

TABLE 3
*Characteristics of the 67 Patients Who Underwent Follow-up Examinations during Treatment**

Characteristic	Total (n = 67)	Standard (n = 17)	Low cholesterol (n = 18)	Bran (n = 16)	MCT (n = 16)
% Wt change (mean ± SD)	+1.07 ± 4.72	+0.46 ± 4.94	−0.14 ± 3.16	+1.06 ± 5.80	+3.08 ± 4.83
Symptoms					
Biliary colic (no of pts)	30	8	6	9	7
Dyspepsia (no. of pts)	31	8	8	9	6
Potential side effects					
Abnormal hepatic chemistries (% of pts)	15.3	23.5	11.1	13.3	13.3
Diarrhea (% of pts)	8.6	5.8	5.2	11.7	12.5
Calcification of GS (no. of pts)	7	2	2	3	0
Serum lipids, mg/dl ($\bar{x}$ ± SD)					
Total chol	242.5 ± 51.0	254.4 ± 57.4	231.9 ± 37.3	240.0 ± 55.1	244.4 ± 54.8
LDL chol	160.8 ± 48.1	162.8 ± 53.2	150.9 ± 38.7	167.1 ± 53.0	163.6 ± 49.7
HDL chol	51.6 ± 15.9	53.8 ± 13.1	53.4 ± 13.9	44.2 ± 15.0	54.7 ± 20.3
Triglycerides	141.2 ± 84.5	162.0 ± 127.8	123.2 ± 48.1	143.1 ± 69.8	137.5 ± 75.0
Bile at 6 or 9 months ($\bar{x}$ ± SD)					
Saturation index	0.83 ± .15	0.86 ± .13	0.81 ± .12	0.77 ± .10	0.90 ± .21
UDCA, % of bile acids	49.1 ± 6.0	48.7 ± 3.7	49.9 ± 6.9	51.2 ± 4.3	46.2 ± 7.7
CDCA, % of bile acids	23.1 ± 3.4	23.2 ± 3.0	21.7 ± 2.4	24.2 ± 3.8	23.7 ± 3.9

* No significant differences were found among the groups.

illnesses unrelated to biliary tract disease. Two of the fourteen patients dropped out of the study after randomization, but before any data during treatment were obtained. All patients in whom data were obtained, however, were included in all analyses.

Characteristics during Treatment. No significant differences were found among the dietary groups in any of the characteristics during treatment (Table 3). Bile became unsaturated during therapy in all patients. The diarrhea always was mild, transient, and resolved spontaneously or with temporary reduction in medication. An abnormality of at least one of the hepatic chemistries was seen in 15.3% of patients (aminotransferases 9.2%, alkaline phosphatase 1.5%, GGT 6.1%, and bilirubin 4.6%). All of these elevations were minor, requiring no change in therapy, and half of them returned to normal prior to completion of therapy.

The mean levels of the serum lipids did not change significantly during therapy in any dietary group. Twenty patients had minor elevations of serum triglycerides during the study; 7 of these returned to normal during treatment. Three patients had minor elevations of serum total cholesterol that have not yet returned to normal on continued therapy. Fourteen patients had minor elevations of LDL cholesterol; 6 of these returned to normal during continued therapy.

Reproducibility of US and OCG. Ultrasonography (US) and oral cholecystogram (OCG) were highly reproducible in the determination of the presence of gallstones and their characteristics (Table 4). Kappa values could not be computed when there was insufficient variation in the data (e.g., radiolucent gallstones and shadowing were present in all patients as a prerequisite for entry into the study).

Agreement between US and OCG. Significant agreement between OCG and US was found for determination of the presence and number of gallstones and the diameter of the largest stone on the two comparisons of the initial study films (Table 5). The two comparisons between OCG and US on the final films agreed only for the determination of the presence of gallstones. When gallstones were absent on OCG, US detected them in 7 of the 13 patients. These stones detected only by US usually were single and less than 1 cm in diameter (mean 7.0 ± 3.7 mm), but always induced distal acoustic shadowing.

Discussion

Efficacy and Safety of UDCA. The frequency of dissolution in this study was comparable to that reported in other trials (1–8). The dose (36, 37) of UDCA and length of treatment probably were optimal (38). The rate of patient loss (20.3%) may have been slightly higher than the 10%–15%

TABLE 4
Reproducibility of Oral Cholecystography (OCG) and Ultrasonography (US)

	US			
	Initial films		Final films	
	K	P	K	P
Presence of GS	NC	—	0.81	<0.001
Shadowing	NC	—	0.65	<0.001
Number	0.89	<0.001	0.77	<0.001
Volume of largest	0.63	<0.001	0.42	<0.001
	OCG			
	Initial films		Final films	
	K	P	K	P
Presence of GS	NC	—	0.94	<0.001
Calcification	NC	—	0.71	<0.001
Floating	0.72	<0.001	NC	—
Number	1.00	<0.001	1.00	<0.001
Volume of largest	0.94	<0.001	0.87	<0.001

K, kappa: value with range from 0 to 1; 1 = complete reproducibility (corrected for chance agreement).

NC, not computed: kappa could not be computed owing to insufficient variation.

generally reported (38). UDCA has been reported to decrease the frequency of dyspepsia and biliary colic (39, 40). Although this was not assessed in this study, the frequency of biliary colic and dyspepsia was high during treatment.

The frequency of diarrhea, abnormalities of hepatic chemistries and serum lipids, development of calcification of gallstones, and the effect of UDCA on biliary lipids were comparable to those reported in other trials (1–8). In this study, gallstone size was the only characteristic that was associated with dissolution. The reported association of dissolution with floating gallstones was not detected in this study; however, the statistical power to detect an association was limited.

Effect of Diets. The relatively small number of patients in each dietary group limited the power to detect statistically significant differences in the rate of dissolution among the dietary groups. No reports have demonstrated that a low-cholesterol diet increases the frequency of complete dissolution of gallstones in patients receiving bile acids. In fact, recently, we found that a diet low in cholesterol (250 mg/day) did not facilitate UDCA-induced augmentation of the pools of total bile acids and UDCA or diminution of the biliary secretion and saturation of cholesterol (41). Even if a further decrease in dietary cholesterol—for example, 100 mg/day as in Northfield's studies (12–14)—were efficacious, it would be impractical for long-term use.

Although MCT decreased biliary saturation in the rhesus monkey (17), it did not influence UDCA-induced changes in the secretion of biliary lipids in humans (41) or, in the present study, influence the rate of UDCA-induced dissolution of gallstones.

The addition of bran to the diet has been reported to have a salutary effect on bile composition (18, 19). Bran, in comparable doses, however, has not been shown to influence UDCA-induced changes in the secretion of biliary lipids (41) and, in this study, did not influence UDCA-induced dissolution of gallstones.

Comparison of OCG and US. This is the first report of a prospective, masked comparison of OCG and US in assessing dissolution of gallstones. Shapero et al (42) reported retrospectively that of 22 patients whose gallstones were apparently completely dissolved according to OCG, 7 were found to have residual small calculi on US. Both these studies, therefore, suggest that US is more sensitive than OCG, although a clinical trial of dissolution in Italy (1) noted that OCG and US had equal sensitivity.

The US and OCG findings were not verified surgically in any of these studies so that the true false positive and negative rates remain unknown. In addition, in our study, most patients had B-mode scans initially and then real-time

TABLE 5
Agreement between Oral Cholecystography and Ultrasonography in Dissolution of Gallstones

	Initial films				Final films			
	Reading 1		Reading 2		Reading 1		Reading 2	
	K	P	K	P	K	P	K	P
Presence of GS	NC	—	NC	—	0.41	<0.001	0.51	<0.001
Number	0.38	<0.001	0.61	<0.001	0.14	0.19 (NS)	0.31	<0.02
Size of largest	0.31	<0.001	0.40	<0.001	0.17	0.14 (NS)	0.13	0.31 (NS)

K, kappa: value with range from 0 to 1; 1 = complete agreement (corrected for chance agreement).

NC, not computed: kappa could not be computed owing to insufficient variation.

scans, a more sensitive and specific ultrasonographic technique, for their subsequent evaluation.

Conclusion and Implications

The diets tested in this study probably have no effect on the degree of biliary desaturation or the frequency of dissolution of gallstones by ursodeoxycholic acid. In the future, trials of the influence of diet on dissolution should be conducted with different regimens.

Ultrasonography probably is more sensitive than oral cholecystogram (OCG) for the determination of dissolution of gallstones and, therefore, might avoid overestimation of the rate of dissolution. Previous trials utilizing only OCG for the assessment of dissolution should be reevaluated. Furthermore, the high rate of recurrence reported after successful dissolution (43) may represent partial dissolution with residual gallstones too small to be detected by OCG. The data in this study did not allow an accurate assessment of partial dissolution by ultrasonography. Future studies of dissolution of gallstones should include OCG initially to assess the eligibility of patients and US during treatment to assess complete dissolution. Cholecystography must be used if partial dissolution is to be assessed during therapy.

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Comparison Between 14″ and 16″ Gauge Needles for Aspiration of Human Oocytes

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Abstract

Human oocyte retrieval by 14″ gauge, 19 cm needle and by the longer, narrower 16″ gauge, 33 cm needle was compared. Aspiration rate of the 14″ gauge needle was 3.0 cc/sec, vs. 1.6 cc/sec with the 16″ gauge needle with the same tubing system and vacuum. Use of the shorter, wider aspiration needle increased the number of retrieved oocytes per patient from 1.8 to 2.9 ($p < 0.05$). The number of oocytes recovered by primary follicular aspiration without irrigation increased from 1.2 to 2.3 ova per patient ($p < 0.05$). We conclude that the 14″ gauge needle is superior to the 16″ gauge needle for oocyte retrieval.

Recovery of human oocytes from preovulatory follicles for in vitro fertilization and embryo transfer is usually accomplished via laparoscopy (1). Sonographic oocyte retrieval is, however, a possible alternative (2–4). Oocytes are recovered by needle puncture of the follicle, followed by aspiration of its contents.

In an attempt to maximize oocyte recovery, some investigators have studied the effects of various stimulation and aspiration techniques on recovery rates (5–8). These studies, however, have not led to standardization of aspiration equipment. Instead, it appears that each group conducting in vitro fertilization and embryo transfer develops or modifies an aspiration device to suit its own needs and criteria. Oocytes are not always aspirated after the first follicular puncture. Follicular irrigation and repeat aspiration are recommended in those instances. Resulting recovery rates vary and depend on many factors, such as the needle, the flush media, the vacuum applied.

Many groups performing in vitro fertilization and embryo transfer use follicular irrigation (3, 8, 9); some do not (5, 7). In some centers, follicles are irrigated only if no oocytes are observed in the original aspirate (3, 10). One group reports that no attempt was made to irrigate any follicle (11), whereas others routinely flush every collapsed follicle (8).

The purpose of this study was to compare the efficacy of two different needles on the aspiration of human ova. A long, narrow 16″ gauge needle was compared to a shorter, wider 14″ gauge needle for differences in oocyte aspiration rates, follicular and peritoneal flushes, and number of ova recovered.

Patients, Materials, and Methods

After informed consent was obtained, 50 women were scheduled for in vitro fertilization and embryo transfer between January 1983 and April 1984. Fifty-six oocyte retrievals were attempted in this period. In 12 (21%) cycles, oocytes were not recovered despite follicular, peritoneal, and needle irrigations.

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Ovarian stimulation was accomplished with human menopausal gonadotropin and/or clomiphene citrate following standard protocols (1, 5). Follicular growth was assessed sonographically with simultaneous measurements of serum estradiol levels. Oocyte retrieval was timed by chorionic gonadotropin injection. Three-puncture laparoscopy was routinely used for oocyte retrieval (1). Five patients with inaccessible ovaries underwent sonographically guided culdocentesis (4).

Oocyte retrieval rates were compared for two aspiration needles. A 16″ gauge 33 cm long uncoated needle was used for follicular aspiration in the first 42 (75%) consecutive women. For the following 14 (25%) cycles, a wider uncoated needle, 14″ gauge and 19 cm long, was used. Both needles were attached to 40–60 cm polyethylene tubing (1.2 mm I.D., 2.5 mm O.D, Becton-Dickinson Co.). The opposite end was attached to a 15-ml culture tube which served as a suction trap. After follicular puncture, aspiration was accomplished using a suction set at −90 mm Hg. Suction pressure was frequently checked, and the aspiration system was rinsed with double-distilled water followed by flush medium. Aspiration rate was measured for both needles with the same suction pressure, solution, and tubing system. A total of 500 ml was aspirated with both needles, aspiration time was measured for each one, and the flow was calculated by dividing 500 ml by the aspiration time. The wider, shorter 14″ gauge needle aspirated 3.0 ml/sec, whereas the 16″ gauge needle aspirated 1.6 ml/sec. These findings are consistent with Poiseuille's law: the quantity of fluid that will flow through a vessel in a given period of time is equal to the velocity of flow times the cross-sectional area according to the following equation:

$$Q = \frac{\Delta P \cdot r^2}{8nl} \cdot \pi r^2$$

where Q is flow, ΔP is pressure gradient, r is radius of the vessel, n is viscosity of the fluid, 1 is the length of the vessel.

Flush media consisted of two types of heparinized solutions (10 units/ml; Sigma Corp.). At the beginning of this study, Dulbecco's phosphate buffered saline (Gibco Labs) was used for irrigation. Later, Ham's F-10 with glutamate solution (Flow Labs), modified with calcium lactate (Calbiochem) and buffered with Hepes (Sigma Corp.) were used for irrigation and aspiration. Follicles were flushed whenever an oocyte was not recovered in a primary aspiration. The collapsed follicle was filled with flush medium using the same aspiration needle and thereafter aspirated into a new culture tube. The majority of follicles were irrigated two times; a few, however, were irrigated one or three times. Culture tubes containing the aspirates were immediately transported to the laboratory. The aspirates were decanted into petri dishes and surveyed for the presence of oocytes. All accessible follicles were punctured regardless of size. Peritoneal irrigation and aspiration was performed whenever oocytes were not recovered in primary follicular puncture, followed by up to three follicular flushes. A peritoneal wash was also performed whenever corpus luteum was found in one of the ovaries.

Student's *t* test was used for statistical analysis of the two groups.

Results

Follicles were accessible and were aspirated in 44 of 56 oocyte retrieval attempts, with a 79% oocyte recovery rate (Table 1). Fourteen (25%) retrievals were performed with the 14″ gauge needle and 42 (75%) with the 16″ gauge needle. Aspiration with the 14″ gauge needle yielded 2.9 oocytes per patient; aspiration with the 16″ gauge needle yielded 1.8 oocytes per patient (Table 1). With the 14″ gauge needle, 78% of primary attempted follicular aspirations yielded ova, whereas only 65% of follicular aspirations with the 16″ gauge needle resulted in recovery of oocytes ($p < 0.05$).

Altogether, 335 follicular flushes were performed: 3.3 flushes per patient with the 14″ gauge needle and 11.3 flushes per patient with the 16″ gauge needle. From follicular and peritoneal flushes, 9 oocytes were recovered with the 14″ gauge needle and 26 with the 16″ gauge needle.

Irrigation and aspiration of the cul de sac and the peritoneal surface around the ovaries yielded recovery of one oocyte in 3.5 flushes with the 14″ gauge needle, and one oocyte in 20.5 peritoneal flushes with the 16″ gauge needle (Table 2). Needle irrigation yielded 1 oocyte per 6.5 flushes of the 14″ gauge needle and one oocyte per 96 flushes of the 16″ gauge needle. Less peritoneal irrigations were necessary with the 14″ gauge needle ($p < 0.01$).

Discussion

Oocyte recovery rates in in vitro fertilization and embryo transfer vary (1–3, 5–11). These variations probably reflect differences in aspiration techniques as well as differences in defining

TABLE 1
Oocyte Retrievals with 14″ and 16″ Gauge Needles

No. of Oocytes	Total	Needle 14″	16″	*p*
Attempted retrievals	56	14.0	42.0	—
Retrieved	116	41.0	75.0	<0.05
Retrieved per patient		2.9	1.8	—
Retrieved by primary follicular aspiration	81	32.0	49.0	<0.05
Retrieved by primary follicular aspiration per patient		2.3	1.2	—

TABLE 2
Results of Peritoneal and Needle Irrigations following Unsuccessful Primary Follicular Oocyte Retrieval

	Total	Needle 14″	16″
No. of peritoneal irrigations and aspirations performed	89	7.0	82.0*
No. of peritoneal irrigations per one retrieved oocyte	—	3.5	20.5
No. of needle irrigations per one retrieved oocyte	—	6.5	96.0

* $p < 0.01$.

recovery rates. Only one group reported 100% oocyte recovery (6). This was accomplished on a small number of follicles using a Teflon-lined needle. Other authors have reported oocyte recovery rates of 75% (11) and 77% (3). The 79% successful oocyte retrieval in the series reported here is comparable to these reports. Small differences in collection rates probably stem from different aspiration equipment, operative techniques, number of follicles per patient, and mode of ovarian stimulation.

Thirty percent of all the recovered oocytes were obtained from irrigation and aspiration of collapsed follicles or from the peritoneal surface. These figures are consistent with other reports (3). However, attempts should be made to minimize that fraction. Some authors suggested high suction pressures of up to −150 mm Hg to improve the recovery rate (5, 7). No data are available on possible mechanical damage to oocytes, either from the high pressure or from collisions on the tubing walls. Nevertheless, high oocyte recovery can be accomplished using suction pressure of less than 120 mm Hg (3, 6, 11); these lower pressures are generally recommended and used (1, 6, 8). In this study, a larger needle was compared with a narrower one with the same relatively low suction of −90 mm Hg. Some authors have reported that a Teflon-lined needle may improve recovery rates by decreasing friction and adherence on the inner walls of the needle (6). Both needles in this study were uncoated; however, the wider 14″ gauge needle was only 19 cm long, compared to the 33 cm length of the 16″ gauge needle, resulting in lower resistance and friction. Increasing the diameter and shortening the needle almost doubled fluid aspiration rate with the same pressure and tubing. According to Poiseuille's law, the flow is reciprocal to the length of the vessel and directly proportional to the fourth power of the radius of the vessel. A small increment in the radius of the needle results in a large increment in the fluid flow. This flow probably detaches the cumulus and results in recovery of the oocyte. Indeed, a change of the needle resulted in an increase of primary oocyte retrieval rate from 1.2 to 2.3 ova per patient ($p <$ 0.05).

Irrigation and aspiration may loosen the still-attached cumulus via mechanical forces; perhaps some of the flush medium components contribute to this process. Use of follicular irrigation has been mentioned by several groups to improve oocyte recovery rates (2, 8, 10). Peritoneal flush is usually performed whenever there are signs of recent ovulation, or whenever oocytes are not recovered in the first attempt and follicular flushes are negative. In this study, peritoneal flush was performed 3.5 times per recovered ovum with the 14″ gauge needle and 20.5 times with the 16″ gauge needle. Furthermore, ova adherent to the needle were easier to recover with the 14″ gauge needle: 6.5 vs 96 needle irrigations per oocyte retrieved from the needle (Table 2).

Use of a shorter, wider needle improves the total number of recovered ova and minimizes the necessity for repeated follicular and peritoneal irrigations. Primary follicular aspiration rate is also improved, and oocytes adherent to the needle are easier to obtain. The 14″ gauge, 19 cm needle is superior to the 16″ gauge, 33 cm needle because it improves the efficacy of human oocyte retrieval.

Summary

Human oocyte retrieval rate was improved when the 16″ gauge, 33 cm long needle was replaced by a 14″ gauge, 19 cm long aspiration needle. The number of oocytes recovered by primary follicular aspiration without irrigation was almost doubled with the larger needle.

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Electrocardiographic Abnormalities in Diabetes Mellitus: Association between T-Wave Changes and Nephropathy

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Abstract

The electrocardiogram was examined using the Minnesota Code in a group of 229 patients aged 30–92 (mean 55.9) with insulin-treated type II diabetes mellitus. Abnormal electrocardiograms were found in 107 of 229 patients (46.7%). Of these, 26 (11%) were diagnosed as myocardial infarction (Minnesota Code 1.1 or 1.2). An additional 16 subjects (7%) had a documented history of myocardial infarction without persistent diagnostic electrocardiographic changes. In the 42 (18%) subjects with myocardial infarction, the mean level of high-density lipoprotein cholesterol was significantly reduced ($p < 0.05$) as compared to the remainder. Left ventricular hypertrophy was present in 22.6% of the 229 subjects, consistent with the prevalence of high blood pressure. T-wave changes were present in 37.6% of the electrocardiograms; these changes strongly correlated with serum creatinine, blood urea nitrogen (BUN), and urine protein levels, but not with low-density or high-density lipoprotein cholesterol or plasma glucose. The high prevalence of T-wave abnormalities was not associated with any other criteria for macrovascular disease. The changes in T-wave conformation remained the largest single category (26.9%) of abnormalities after all subjects with myocardial infarction or LVH were eliminated.

The 86 patients with abnormal T waves had a serum creatinine level of 1.41 ± 0.14 mg/dl, and a BUN of 21.6 ± 1.01 mg/dl, whereas the 143 patients with normal T waves had a serum creatinine level of 1.02 ± 0.02 mg/dl, and a BUN level of 17.4 ± 0.49 mg/dl. Both differences were highly statistically significant ($p < 0.001$). This strong association of T-wave changes with changes of renal function (without renal failure) suggested that the underlying abnormality may be microvascular in origin.

Electrocardiographic abnormalities have been reported in more than 40% of type II diabetics (1). On the basis of the Minnesota Code criteria, a high prevalence of abnormalities may be found in the categories of ST segment, T, and Q waves. The frequency of electrocardiographic abnormalities appears to be greater than that predicted by the increased prevalence of coronary heart disease or high blood pressure in diabetics. Furthermore, such ST-T wave changes are found in a variety of other cardiac disorders, such as metabolic derangements or myocardial disease. Autopsy studies have shown that some diabetics have small-vessel disease and/or glycoprotein deposition among myocardial muscle fibers (2). Interstitial fibrosis has been described in the heart of the diabetic hypertensive rat (3). Physiologic studies in a small number of patients have revealed decreased ejection fraction suggesting myocardial dysfunction (4).

In this article we have attempted to systematically relate a variety of clinical parameters to the abnormalities in the electrocardiogram as classified by the Minnesota Code. In a group of insulin-treated, middle-aged subjects with long-

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standing diabetes (mean duration 15 years), those with definite coronary heart disease (CHD) have been contrasted to a larger group with EKG changes for which there is no clinical evidence of such disease.

Patients and Methods

The master list for this study included the 750 patients who constituted the total insulin-treated population attending the Diabetes Clinic of The Mount Sinai Medical Center as of September 1, 1979. Patients are mostly members of the inner-city minority groups, blacks and Latins, representing low-income families who live in the immediate surrounding and neighboring areas of New York City. The group encompasses those patients who are average diabetics, insulin dependent, comparable in age and sex, and who offer an adequate sample for this general study. Contacts were attempted with all patients who had arrived in our clinic through referrals from the emergency room and from other clinics and doctors. Completed interviews numbered 550; 430 persons volunteered to return for complete physical examinations and laboratory analyses, but the other 120 did not agree to participate; 268 patients were willing to remain to have an EKG done on the afternoon of the day of the initial examination. Electrocardiograms were taken at least 2 hr after the breakfast meal.

Patients were excluded if they had type I diabetes ($n = 24$) by the criteria of having experienced ketoacidosis at least once and/or having required insulin treatment beginning within one month of the diagnosis of diabetes. An additional 15 patients were excluded owing to missing data. The final cohort for analysis was 229 patients. This group represents 30.5% of the 750 total. Thus, the population studied may not be cross-sectionally representative of the total number of diabetic patients in the clinic, but there is no reason to question the validity of the conclusions based on the cohort. The percentage is still a highly relevant sample for a study. Exclusion of the 39 subjects did not change the conclusions or the level of statistical significance of any of the analyses.

This subset of 229 patients was representative of the cohort of all diabetic volunteers (Table 1). Not only were they similar in the characteristics given in Table 1, but they were found to have no significant differences in the use of the following medication: diuretics, antihypertensives, digitalis preparations, and potassium supplementation. Data sets from these patients, were recorded and analyzed using the Statistical Analysis System (SAS) program in the City Univeristy of New York Computer Center.

TABLE 1

Comparison of EKG of Sampled with Nonsampled Group

	EKG ($n = 229$)	EKG not done ($n = 166$)
Glucose, mg/dl	198.9 ± 91.5	193.6 ± 93.0
Systolic BP, mm Hg	147.0 ± 23.3	144.3 ± 25.5
Diastolic BP, mm Hg	84.1 ± 11.2	83.4 ± 12.8
Body mass index, kg/m^2	30.6 ± 6.4	29.2 ± 5.6
Creatinine, mg/dl	1.2 ± .8	1.1 ± .5
BUN, mg/dl	18.9 ± 7.6	17.9 ± 6.7
Cholesterol, mg/dl	209.3 ± 47.6	210.0 ± 47.2
HDL cholesterol, mg/dl	44.1 ± 13.4	46.8 ± 14.6
Triglycerides, mg/dl	135.3 ± 77.1	127.7 ± 67.5
Age, yrs	55.9 ± 11.5	58.6 ± 13.5
HB, AIC, %	6.5 ± 2.0	6.6 ± 2.0
Insulin dose, units/day	48.3 ± 28.9	44.2 ± 28.2
Duration of diabetes, yrs	15.3 ± 8.0	13.8 ± 8.2

Values are mean and standard deviation. No difference between the groups is significant by t test at $p < 0.05$.

More detailed information regarding the acquisition of the cohort and the clinical and demographic characteristics of the group has been previously reported (5).

Blood pressure was measured with a mercury column sphygmomanometer in the left arm with the patient in the supine position. Diastolic pressure was measured by the Korotkoff V disappearance of the sound. All patients had a resting 12-lead EKG analyzed according to Minnesota Code (6). All the items of the Minnesota Code were considered, including abnorm criteria 5.3, S junctions from 4.1 to 4.3, Q and QS patterns from 1.1 to 1.3. However, electrocardiographic criteria for myocardial infarction were considered met only if 1.1 or 1.2 was present, since 1.3 correlates poorly with infarction (7). A blood sample obtained after overnight fasting was analyzed for lipids, lipoproteins, plasma glucose, hemoglobin A1C, and other routine chemistries. Creatinine was measured by the Jaffe method (8). The urine was examined for protein and glucose using enzymatic techniques with an ABA 100 Autoanalyzer (Abbott Laboratories). The HDL cholesterol was determined after precipitation of all other lipoproteins using $MgCl_2$/dextran sulfate (9). The LDL cholesterol was estimated by the method of Fridewald et al (10): total cholesterol minus HDL cholesterol minus one-fifth of the triglyceride. The statistical analysis used Student's t test, Pearson's correlation coefficient, analysis of variance, analysis of covariance, and the chi-square method, whenever applicable.

Results

Abnormalities in the EKG were found in 107 of 229 patients (46%). For this analysis the subjects were divided into those with myocardial infarction and those with ventricular hypertrophy. EKG changes diagnostic of previous myocardial infarction (Minnesota Code criterion 1.1 or 1.2) were present in 26 patients (11.4%), but only 5 of these had a history of myocardial infarction (Table 2). Thus, 21 subjects met the EKG criteria without giving a history of an acute event. Sixteen individuals had a history of myocardial infarction (Table 2) without persistent diagnostic EKG changes. In those subjects with a history of myocardial infarction as the only criterion, the EKG was usually abnormal, having T-wave or ST-segment changes.

Compared to the remainder of the cohort, the HDL cholesterol in the total group of 42 patients with myocardial infarction (MI) was significantly lower. This difference in HDL persisted when the subgroups having MI by EKG criteria only or by history only were compared to the remainder of the group. This difference was noted with the group as a whole when the comparison was made among males only ($p < 0.01$). HDL cholesterol was 41.6 ± 13.0 in those without MI, but this was not significant. No other risk factor was found to significantly differentiate between the myocardial infarction group (Table 3) and those without evidence of MI in this small number of individuals. There was no difference in the smoking prevalence, blood pressure, fasting plasma glucose levels, Hgb AlC, or duration of diabetes in subjects with or without myocardial infarction.

T-wave changes with or without ST changes occurred in 86/229 patients (37.6%), making those the most common abnormality found (Tables 4, 5). When the 187 patients without myocardial infarction (by history or EKG criteria) were considered, the frequency of T-wave changes was 29.9%. LVH was found in 51 of the 229 patients. When subjects with LVH and/or myocardial infarction were excluded, the prevalence of T-wave changes was 26.9% of the 156 remaining patients (Table 5).

Several parameters were significantly different in individuals with T-wave abnormalities as compared to the remainder of the group, including significantly higher serum creatine, BUN, and blood pressure (Table 6). There was also a higher incidence of proteinuria. When the total group ($n = 229$) was examined, 29.4% of patients with abnormal T waves had proteinuria, whereas only 16.7% of patients without T-wave abnormalities had detectable urine protein (1+ or greater). This was statistically significant ($p < 0.05$). When subjects without MI or LVH were deleted from the analysis, those with T-wave abnormalities had 25.6% proteinuria, a significantly higher value ($p < 0.05$) than the 15.7% in those with normal T waves.

TABLE 2

Prevalence of Myocardial Infarction in 229 Diabetic Patients

	Women no.	Men no.	Total no. (%)
Total	155	74	229 (100)
MI by EKG*	14	12	26 (11.4)
MI by history†	14	7	21 (9.2)
Both‡	2	3	5 (2.2)
Total	26	16	42 (18.3)

* Electrocardiographically detectable myocardial infarction.

†History of myocardial infarction.

‡Historical and electrocardiographically detectable myocardial infarction.

Potassium levels were not significantly different in the two groups. Only 10 individuals had potassium levels below 3.55 mEq/liter, and when these were eliminated, there was no change in the association of nephropathy and T-wave abnormalities.

The strong relationship betweeen T-wave abnormalities and indices of diminished renal function (creatinine, BUN, and proteinuria) might be explained by the higher level of blood pressure in subjects with T-wave abnormalities. The frequency of hypertension among those individuals was significantly higher, since 65 of 86 (76%) subjects with T-wave abnormalities had blood pressure levels greater than 140 systolic, whereas 82 individuals (57%) of the 143 with normal T waves had blood pressure greater than this value. An initial attempt to examine this question was made by using covariate analysis in which creatinine levels in the two groups were examined after controlling for blood pressure. The unadjusted mean creatinine levels in those patients with normal and abnormal T waves were 1.02 and 1.43 mg/dl, respectively. After adjustment with the covariate analytic procedure, the mean values were 1.03 and 1.42 mg/dl, respectively. Thus, this correction did not change the relative creatinine level, and the difference remained highly significant by statistical tests. Therefore, the relationship between the creatinine and blood pressure levels did not account for the higher creatinine levels in those patients with T-wave abnormalities.

TABLE 3
Selected Characteristics of Subjects with Myocardial Infarction
(Mean ± SD by t-test)

	HMI		EMI		Total MI	
	Yes (n = 21)	No (n = 208)	Yes (n = 26)	No (n = 203)	Yes (n = 42)	No (n = 187)
HDL (mg/dl)	38.2 ± 11	448 ± 14*	413 ± 12	445 ± 14	39.6 ± 11	45.1 ± 14**
LDL (mg/dl)	131.0 ± 44	136.0 ± 39	140.0 ± 53	136.0 ± 39	139.0 ± 45	135.0 ± 39
TGL (mg/dl)	159.0 ± 100	131.0 ± 68	137.0 ± 56	135.0 ± 79	152.0 ± 80	129.0 ± 69
TC (mg/dl)	216.0 ± 53	208.0 ± 46	227.0 ± 69	207.0 ± 44	220.0 ± 59	206.0 ± 44
PG (mg/dl)	206.0 ± 86	188.0 ± 79	191.0 ± 82	189.0 ± 80	199.0 ± 79	187.0 ± 80
AIC (%)	7.0 ± 2	6.5 ± 2	6.2 ± 2	6.5 ± 2	6.7 ± 2	6.4 ± 2
D (yrs)	16.0 ± 7	15.0 ± 8	15.0 ± 10	15.0 ± 8	16.0 ± 9	15.0 ± 8
Age (yrs)	60.0 ± 10	60.0 ± 12	62.0 ± 9	60.0 ± 12	62.0 ± 10	62.0 ± 9
SBP (mm Hg)	153.0 ± 24	146.0 ± 23	149.0 ± 29	147.0 ± 23	149.0 ± 26	146.0 ± 23
DBP (mm Hg)	84.0 ± 10	84.0 ± 12	89.0 ± 12	84.0 ± 11	84.0 ± 11	84.0 ± 11

HMI, history of myocardial infarction; EMI, electrocardiographic evidence of myocardial infarction; MI, myocardial infarction; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TGL, triglycerides; TC, total cholesterol; PG, plasma glucose; AIC, hemoglobin AIC; D, duration of diabetes; SBP, systolic blood pressure; DBP, diastolic blood pressure.

* $p < 0.05$.

** $p < 0.005$. Five patients had both HMI and MI.

Another possibility is that those individuals with abnormal renal function used some drug, or drugs, which induced the T-wave abnormalities. We examined for this possibility by eliminating all subjects on diuretics, antihypertensive agents, and digitalis preparations. When the 43 subjects with T-wave abnormalities were compared with the 107 subjects with normal T waves after this procedure, the mean creatinine level remained highly significantly different at 1.42 ± 1.5 mg/dl versus 0.99 ± 0.3 mg/dl ($p < 0.007$).

Of the 229 patients analyzed, 155 were female and 74 were male. The frequency of EKG abnormalities was not statistically different between the sexes. History of myocardial infarction had occurred with identical frequency in males and females (9.7% versus 9.5%). The lack of male-female differences was less surprising in view of the higher level of risk factors for cardiovascular disease in the women. They had significantly higher systolic blood pressure (150 ± 25 mm/Hg versus 140 ± 19 mm/Hg) and a more frequent history of hypertension (53% versus 29%), and received higher doses of insulin (52 ± 30 units/day versus 41 ± 25 units/day). Obesity was more frequent in females, as indicated by the Quetelet index (32 ± 7 kg/m in women versus 28 ± 5 kg/m in men). The mean values were in the 90th percentile for women and the 75th percentile for men, as measured in a free-living population (11). The fasting plasma glucose was also higher in females (212 ± 89 mg/dl versus 169 ± 88 mg/dl). Males smoked more cigarettes and consumed more alcohol. Males also had higher

TABLE 4
Prevalence of EKG Abnormalities
(N = 229)

Abnormality*	Female (n = 155)	Male (n = 74)	Total No. (%)
Any abnormality	71	36	107 (47)
T-wave changes	60	26	86 (38)
Left ventricular hypertrophy	29	22	51 (22)
Q†	14	12	26 (11)
ST depression	18	6	24 (10)

* Prevalence of each abnormality with or without other electrocardiographic abnormalities, except for ST depression, which requires a T-wave code.

† Q_1 or Q_2 items as specified in Minnesota Code (6).

TABLE 5
Prevalence of Specific T-Wave and ST-Segment Abnormality in Type II Diabetes

Minnesota Code assignment	All patients N (%)	Deleting MI N (%)	Deleting MI and LVH N (%)
T-wave changes	86 (37.6)	56 (29.9)	42 (26.9)
5.1	3 (1.3)	1 (0.5)	0 (0)
5.2	22 (9.6)	11 (5.9)	6 (3.8)
5.3	61 (26.6)	44 (23.5)	36 (26.1)
ST depression	24 (10.5)	10 (5.3)	6 (3.8)
4.1	6 (2.6)	2 (1.0)	1 (0.6)
4.2	5 (2.2)	2 (1.0)	1 (0.6)
4.3	13 (5.7)	5 (2.7)	4 (2.6)
Total	229 (100)	187 (100)	157 (100)

Specific Minnesota Code assignments for T-wave changes (5.1, 5.2, 5.3) and ST depression (4.1, 4.2, 4.3) are defined in Ref. 6.

TABLE 6
Electrocardiographic Abnormalities and Kidney Function Parameters

	N	Creatinine	BUN
Total T waves	229		
Abnormal T waves	86	1.41 ± 0.14*	21.6 ± 1.01*
Normal T waves	143	1.02 ± 0.02	17.4 ± 0.49
Deleting MI	187		
Abnormal T waves	56	1.56 ± 0.21*	22.93 ± 1.39*
Normal T waves	131	1.00 ± 0.29	17.10 ± 0.53
Deleting MI and LVH	156		
Abnormal T waves	42	1.41 ± 0.15*	22.33 ± 1.41
Normal T waves	114	0.99 ± 0.03	16.96 ± 0.53

BUN, blood urea nitrogen; *n*, number of patients; MI, myocardial infarction; LVH, left ventricular hypertrophy.

* $p < 0.001$ by *t*-test analysis.

serum creatinine (1.4 ± 1.2 mg/dl versus 1.1 ± 0.6 mg/dl).

Discussion

We found abnormal T waves in the EKG in 38% of 229 insulin-treated type II diabetic patients. This EKG finding may have prognostic significance; in the Prudential Insurance Co. report, more than twice the incidence of seven-year mortality was observed in adult men with T-wave changes as compared with those without (12). In the Framingham study also, T-wave changes were highly predictive of the subsequent development of heart disease (13).

The World Health Organization study reported by Kelly West et al contains the only data set reporting frequency of T-wave abnormalities as determined by Minnesota Code criteria in a similarly large population of diabetic patients (14). The prevalence of T-wave abnormalities ranges up to 30% in the female population (15) and is thus similar to the prevalence in our study. The slightly higher occurrence of T-wave abnormalities in our population may relate to the large number of blacks (42%) and Hispanic subjects (48%) present in this population. Unfortunately, there are no published studies of black and Hispanic diabetics to which these data may be compared. Another potential difference is that in our study all patients had been selected for insulin treatment, whereas in other studies (14), a mixture of therapeutic regimens prevailed. Another factor that may explain the higher prevalence of T-wave changes in our study may be the older age of our patients (30–92 years) compared to patients in the WHO study (33–55 years).

The strong relationship between T-wave abnormalities and blood pressure elevation found in this study suggests that the changes in the heart may, in fact, be secondary to hypertension. It also seems possible that elevations in blood pressure may have produced abnormal renal function, as manifested by the increased creatinine, BUN, and proteinuria found in subjects with T-wave abnormalities. However, this does not appear to be a sufficient explanation, for when the subset of subjects who had elevated blood pressure at the time of the examination or a history of hypertension was eliminated, creatinine remained significantly higher in the normotensive subjects with T-wave abnormalities. When the creatinine was adjusted by covariate analysis for its relationship with blood pressure, a marked difference in the level of creatinine in the group with abnormal T waves persisted. Thus, it seems unlikely that hypertension alone explains the strong relationship between T-wave changes and blood creatinine. The second possibility is that the renal disease was the primary event leading to hypertension which subsequently altered the heart, producing T-wave abnormalities. The frequency of T-wave abnormalities in the nonhypertensive group argues strongly against this hypothesis as the total explanation for the high frequency of the T-wave abnormality. A third possibility is that a primary process leads to the changes in the clinical parameters measured in this study.

There seems to be little doubt that the glycoprotein deposition found in the mesangium at the basement membrane of the glomerular capillaries is associated specifically with diabetes mellitus with or without the coincidence of hypertension. Recently, histologic examination of the diabetic heart has indicated the presence of microvascular changes, including microaneurysms (16), and—perhaps more important—the presence of glycoprotein deposition among

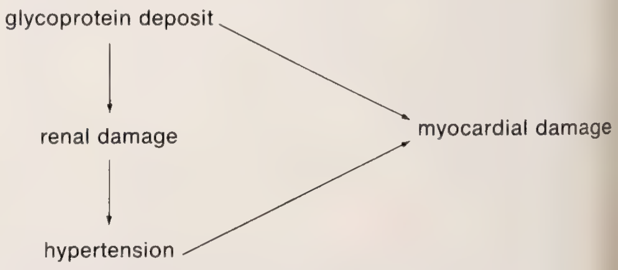

Fig. Possible mechanism of interaction between renal and myocardial damage in diabetes mellitus.

the muscle fibers of the myocardium (2). It has also been noted that the microvascular changes in the heart may be markedly accentuated by the coexistence of hypertension in the diabetic. In a rat model of this disorder, it was only when hypertension was present in the streptozotocin-induced diabetic state that microvasular disease developed in the myocardium (3). To date, autopsy studies have not adequately addressed the possibility that the same pathologic process affecting kidney and myocardium in the diabetic may progress in parallel.

Another possibility is that the relationship between renal dysfunction and T-wave changes may be another expression of arteriosclerotic cardiovascular disease. As expected, a high frequency of myocardial infarction existed within this population of older diabetic patients, 18.3% of the 229 individuals having either a history of myocardial infarction or electrocardiographic changes diagnostic for MI by Minnesota Code criteria. As noted in other studies (13), the prevalence of MI in females was equal to that in males; 26 of the 155 females (16.8%) and 16 of 76 males (21.6%) met criteria for MI. Within the group of subjects having myocardial infarction, HDL levels were significantly reduced (39.6 ± 11.4, as compared to the remainder of the group, 45.1 ± 13.8). However, no other blood chemistry parameter correlated with MI.

The lack of relationship between the prevalence of MI and parameters of renal function (creatinine, BUN, and proteinuria) as well as the weaker relationship with blood pressure clearly distinguishes the survivors of definite MI from those with T-wave abnormalities alone.

It is hypothesized that the T-wave abnormalities are related to the same process that produces renal changes in the diabetic state. Although hypertension may be part of this process, these data indicate that this is not the total explanation and raise the possibility that histopathologic changes in the myocardium and kidney may precede and accompany elevations in blood pressure.

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Exercise-Induced Regional Wall Motion Abnormalities: How Specific a Marker for Coronary Artery Disease?

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Abstract

Exercise radionuclide ventriculography is a relatively sensitive noninvasive technique commonly used to detect coronary artery disease. There is conflicting data regarding the specificity of exercise-induced asynergy for coronary artery disease. We report the case of a patient with dilated cardiomyopathy and exercise-induced deterioration of regional wall motion. We review clinical entities other than obstructive coronary artery disease and discuss additional mechanisms that may play a role in the development of exercise-induced asynergy demonstrated by radionuclide ventriculography.

Regional myocardial dysfunction induced by experimental coronary occlusion was originally described by Tennant and Wiggers (1) more than forty years ago. Herman et al documented the close relationship between regional asynergy detected by contrast ventriculography and obstructive coronary artery lesions (2). The detection of regional wall motion abnormalities as well as the determination of left ventricular ejection fraction are now commonly determined noninvasively by radionuclide ventriculography (3, 4). Good correlation between the results of radionuclide ventriculography and traditional contrast angiography has been confirmed by numerous investigators (5, 6). In addition to resting wall motion abnormalities, stress-induced regional asynergy has been documented during contrast angiography with atrial pacing in patients with coronary artery disease (7). In response to the need for a noninvasive screening test for coronary artery disease, exercise radionuclide ventriculography has emerged as a relatively sensitive noninvasive technique capable of detecting regional asynergy (8).

For purposes of discussion we report a case of a patient with dilated cardiomyopathy and exercise-induced deterioration of regional wall motion. There is conflicting data concerning the specificity of exercise radionuclide ventriculography for coronary artery disease. Mechanisms other than obstructive coronary artery disease may be involved in the development of exercise-induced asynergy occurring both in patients with known cardiomyopathies and in "normal" persons.

Case Report

A 36-year-old Hispanic man with a twenty-year history of essential hypertension and long history of alcohol abuse consulted us for a cerebrovascular accident and poorly controlled hypertension in 1982. The patient reported no dyspnea, chest pain, or past myocardial infarction. He did report a history of gout and a family history of coronary artery disease. Cardiomegaly was noted at that time. The electrocardiogram showed normal sinus rhythm, biatrial enlargement, nonspecific intraventricular conduction delay with

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secondary repolarization abnormality, and small q waves in the inferior leads of uncertain significance. Echocardiography revealed left ventricular, right ventricular, and left atrial enlargement, mitral valve prolapse, and severe diffuse left ventricular dysfunction. The left ventricular ejection fraction at rest was 13% as determined by gated blood pool ventriculography. The patient was treated with warfarin, furosemide, digoxin, and minoxidil. He was readmitted in October 1982 with a right intracerebral hematoma. Warfarin was discontinued, and the patient showed good neurologic recovery. The patient was subsequently followed in clinic.

The patient was referred in June 1983 for a gated blood pool study. The resting ejection fraction was now 35% with diffuse hypokinesis involving all segments and a small area of localized apical akinesis. Exercise was performed to determine whether reversible segmental dysfunction was present and to further elucidate the etiology of his cardiac disease. With fatigue-limited supine bicycle exercise to 75 watts, there was now severe diffuse left ventricular dysfunction with a decrease in ejection fraction to 23% (Fig.). In addition, there were new areas of inferoapical akinesis and more severe septal hypokinesis.

In view of the presence of risk factors for coronary artery disease and exercise-induced asynergy, the patient underwent right and left heart catheterization for study of the hemodynamics, review of the left ventricular angiogram, and ruling out of coronary artery disease. The coronary arteries were angiographically normal. The right anterior oblique left ventriculogram showed enlarged systolic and diastolic dimensions and confirmed the presence of regional asynergy at rest. There was severe hypokinesis of the diaphragmatic wall, moderate hypokinesis of the anterolateral wall, and apical akinesis present.

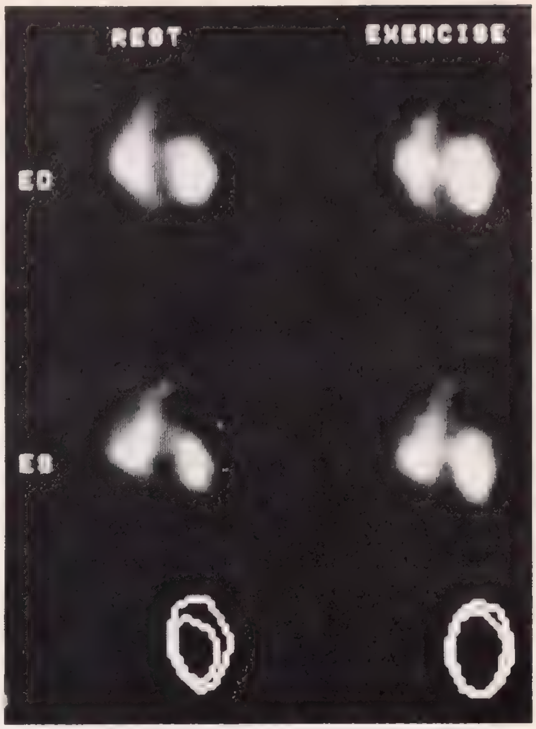

Fig. Left anterior oblique gated blood pool images of patient with cardiomyopathy at rest (left) and with exercise (right). Outlines (below) represent end systolic edges superimposed on end diastolic edges for rest (left) and exercise (right). ED, end diastole; ES, end systole.

Discussion

Specificity of exercise-induced asynergy for coronary artery disease. The literature on the specificity of exercise-induced regional wall motion abnormalities for the diagnosis of coronary artery disease has been contradictory. Multiple studies (8–16) have reported the presence of exercise-induced regional wall motion abnormalities detected by stress radionuclide angiography to be 100% specific for the diagnosis of coronary artery disease (Table 1). Borer et al studied 14 normals without electrocardiographic or clinical evidence of cardiovascular disease. With supine bicycle exercise, all of the normals increased their ejection fraction, and no patients developed regional wall motion abnormalities (8). Borer et al subsequently reported on 21 patients with chest pain and angiographically normal coronary arteries and found no regional dysfunction with exercise, concluding the specificity was 100% for coronary artery disease (11). Jengo et al reported normal segmental wall motion during upright bicycle exercise in all 7 of their patients with normal or insignificantly obstructed coronary arteries (13).

In these studies, regional wall motion abnormalities were found to be highly specific for coronary artery disease. The control groups in these consisted of clinically normal and/or angiographically proven normal patients.

Other investigators (17–22), however, have reported both abnormal stress ejection fraction responses and exercise-induced regional wall motion abnormalities in angiographically normal patients without coronary artery disease (Table 1). Rozanski et al (17) evaluated 17 consecutive patients with normal coronary angiograms and reported a 53% incidence of regional wall motion abnormalities induced by upright bicycle exercise. Although Berger et al (14) initially reported 13 normal individuals with normal rest and exer-

TABLE 1

Studies Evaluating Specificity of Exercise Induced Asynergy for Coronary Artery Disease

Investigator	Ex-asyn: Normals*	Specificity†	Ex-asyn: CAD‡	How normals identified	Method	Stress Intervention
Borer 1977 (8)	0/14	100%	11/11	clinical history	GBPI	supine bicycle
Rerych 1978 (9)	0/30	100%	24/30	clinical history	first pass	upright bicycle
Borer 1979 (11)	0/21	100%	59/63	contrast angiography	GBPI	supine bicycle
Wainright 1979 (10)	0/22	100%	—	contrast angiography	GBPI	cold pressor
Berger 1979 (14)	0/13	100%	28/60	5:contrast angiography 8: clinical history	first pass	upright bicycle
Jengo 1979 (13)	0/7	100%	11/11	contrast angiography	first pass	upright bicycle
Massie 1979 (12)	0/6	100%	10/10	contrast angiography	GBPI	—
Brady 1979 (15)	0/19	100%	52/70	contrast angiography	GBPI	supine bicycle
Bodenheimer 1979 (20)	1/30	97%	66/98	contrast angiography	first pass	handgrip
Berger 1981 (18)	4/31	87%	—	exercise ECG	first pass	upright bicycle
Gibbons 1981 (19)	4/60	93%	—	contrast angiography	first pass	upright bicycle
Jones 1981 (21)	3/50	94%	121/229	contrast angiography	first pass	upright bicycle
Rozanski 1981 (17)	9/17	47%	—	contrast angiography	GBPI	upright bicycle
Osbakken 1984 (22)	6/34	82%	37/86	contrast angiography	GBPI	supine bicycle

GBPI, gated blood pool imaging.

* Number of normal subjects developing exercise-induced asynergy.

† $\dfrac{\text{True normals detected}}{\text{Total number of normals}}$

‡ Number of patients with coronary artery disease developing exercise-induced asynergy.

cise wall motion, the same group of authors (18) subsequently reported 31 patients with chest pain syndromes, ischemic-appearing exercise electrocardiograms, and angiographically normal coronary arteries, finding 39% with an abnormal ejection fraction response to exercise. Four of these patients (13%) developed regional wall motion abnormalities with exercise, each of them showing an abnormal global response as well. Most recently, Osbakken et al (22) reported an 18% incidence of exercise-induced asynergy in the absence of coronary artery disease.

Thus data on the specificity of radionuclide evaluation of exercise-induced wall motion abnormality for obstructive coronary artery disease conflict, possibly influenced by different or changing "normal" populations; an increasing number of investigators are uncovering abnormal wall motion responses in "normal" individuals. There may be several reasons for this apparent change in specificity. Rozanski et al (23) report a decline in the specificity of radionuclide angiography from 1978 to 1982 attributable to a changing pattern of patient referral. Previously, exercise gated blood pool studies were performed for research purposes in addition to catheterization. In recent years, gated blood pool imaging has been used primarily as a screening test for the catheterization laboratory. Selected patients with positive radionuclide tests are now referred for angiography on the basis of the noninvasive study.

In addition to changing patterns of patient referral, other pathophysiologic mechanisms are involved in the occurrence of resting or exercise-induced asynergy in patients without coronary artery disease.

Other Pathophysiologic Mechanisms Although the classic mechanisms of limited supply and increased demand due to obstructive coronary artery disease account for the majority of cases of exercise-induced asynergy, other pathophysiologic mechanisms are now being recognized.

Coronary vasomotor abnormalities. Increased coronary artery tone manifested by either epicardial coronary artery spasm or abnormal arteriolar vasodilator reserve has been proposed as one alternate explanation. Fuller et al (24) describe two patients with exercise-induced regional asynergy determined by radionuclide ventriculography, and exercise-induced coronary spasm documented by contrast angiography. Cannon et al report a 33% incidence of exercise-induced regional dysfunction in patients with normal epicardial coronary arteries but with an abnormal arteriolar vasodilator response to pacing (25, 26). Ergonovine unmasked or exacerbated these abnormalities in arteriolar vasodilator reserve (25). Patients with idiopathic dilated cardiomyopathy may have impaired coronary dilatory capacity unmasked during dipyridamole challenge (27), although this mechanism has not been shown to account for exercise-induced asynergy in this patient group. Although defects in vascular tone may be an occasional cause, other mechanisms must also be involved in the absence of coronary artery disease, since Berger et al (18) demonstrated a negative ergonovine challenge in angiographically normal patients with exercise-induced asynergy.

Primary myocardial disease. It is not yet clear whether abnormal regional and/or global exercise responses in otherwise normal patients without coronary artery disease do in fact represent occult myocardial disease. We describe a patient with a dilated cardiomyopathy who manifests both global and regional abnormalities in left ventricular function both at rest and induced by exercise. The exercise response in patients with known cardiomyopathy has only recently been studied. Our patient's development of marked global and regional deterioration in left ventricular function is not consistent with the results of the study of Higginbotham et al, who report on 48 patients with idiopathic cardiomyopathy who develop no regional wall motion abnormalities with exercise (28). In that study, however, patients with chest pain syndromes or abnormal ST responses during exercise were excluded.

Other noncoronary etiologies. It is well accepted that nonischemic etiologies of cardiomyopathy may result in resting regional wall motion abnormalities. Regional asynergy at rest has been described in mitral valve prolapse (29), Coxsackie B5 myopericarditis (30, 31), idiopathic dilated cardiomyopathy (28, 32) Chagas' disease (33), sarcoid heart disease (34), acute myocarditis (35), Uhl's disease (36), beta thalassemia with chronic iron overload (37), small-vessel disease (38), necrosis secondary to abnormal hemoglobin-oxygen dissociation curves (39), and therapy with doxorubicin (40). Gated blood pool studies at rest have been used for differentiation of idiopathic congestive cardiomyopathy from the left ventricular dysfunction of coronary artery disease. However, because of the occurrence of regional asynergy at rest in nonischemic cardiomyopathy, this assessment can be quite difficult (41–43). Bulkley et al found that left ventricular segmental wall motion abnormalities at rest could not reliably differentiate ischemic cardiomyopathy from idiopathic dilated cardiomyopathy (43).

TABLE 2

Exercise-Induced Asynergy in Disease other than Obstructive Coronary Artery Disease

Investigator	EI asyn	Disorder	Method	Stress
Hecht (48)	8/19	valvular heart disease	GBPI	SB
Leon (36)	1/24	β-thalassemia + chronic iron overload	GBPI	SB
Fuller (24)	2/2	exercise-induced coronary spasm	GBPI, CA	SB
Cannon (26)	8/24	abnormal arteriolar reserve	GBPI, CA	SB
Follanbee (46)	7/26	sclerodema	GBPI	SB

EI asyn, exercise-induced asynergy; GBPI, gated blood pool imaging; CA, contrast angiography; SB, supine bicycle.

The common pathologic denominator for irreversible segmental asynergy of both coronary artery disease and many noncoronary myocardial diseases appears to be myocardial fibrosis. Resting regional dysfunction may occur secondarily to localized cardiomyopathic processes with focal fibrosis (44). Losse reported abnormal right ventricular biopsies in all 12 patients with thallium 201 defects and normal coronaries (45). Resting segmental abnormalities therefore are clearly not specific for coronary artery disease.

Abnormal ejection fraction response with exercise is also not specific for coronary artery disease. Stress-induced global dysfunction has been described in healthy volunteers of advanced age (46), scleroderma (47), valvular heart disease, hypertrophic cardiomyopathy, dilated cardiomyopathy, mitral valve prolapse, and clinically normal subjects (48).

Regional wall motion abnormalities induced by exercise have also been reported in several clinical entities other than coronary artery disease (Table 2). Stress-related segmental abnormalities were reported by Hecht et al (49) in 42% of patients with valvular heart disease, aortic regurgitation being the most common lesion. These patients most often developed inferoapical abnormalities with exercise. Patients with aortic regurgitation may have an exercise-induced fall in ejection fraction and asynergy due to abnormal peak systolic wall stress (50). Experimentally, acute elevation of wall stress by partial aortic occlusion has produced apical asynergy in the dog model (51). Evaluation of the myocardial ultrastructure in patients with chronic aortic regurgitation has shown loss of myofibrils, interstitial fibrosis, and varying degrees of degeneration of cardiac muscle cells (52). Due to the presence of fibrosis, the true amount of viable myocardium in a segment may be reduced despite normal wall thickness measured by echocardiography.

In cases similar to the one presented, abnormal segmental wall motion responses may be due to partial focal fibrosis or localized cardiomyopathic segments. Normal myocardial reserve necessary for a rise in ejection fraction with exercise and preservation of regional function requires not only epicardial and small vessels of normal caliber and tone, but also normal ventricular wall stress (50) and histologically healthy myocardium. In a ventricular segment with abnormal wall stress, it is reasonable to postulate a reduction in the transmyocardial perfusion gradient, resulting in subendocardial ischemia and asynergy in the absence of coronary artery disease. In patients with abnormal regional wall stress, subendocardial ischemia may also be responsible for the "false positive" ST segment response during exercise in some of these individuals.

It is possible that differences in left ventricular geometry that occur with cardiomyopathies may influence the presence or absence of exercise-induced asynergy in cardiomyopathy. For instance, the globular, dilated left ventricle of mitral insufficiency may distribute intracavitary pressures more uniformly than the prolate ellipsoid shape retained in aortic insufficiency.

How often is exercise-induced asynergy a technical artifact? One potential question concerning the increasing incidence of exercise-induced asynergy without obstructive coronary artery disease is whether methodologic differences exist between conflicting studies. Although a theoretical difference may exist between upright or supine bicycle exercise for the detection of asynergy, review of the published studies (Table 1) illustrates that laboratories employing one or the other technique have demonstrated a range of specificities without a clear pattern.

Another possible cause of exercise-induced asynergy in patients without coronary artery disease may be an artifact of the gating technique. Due to the reliance of gated blood pool imaging on a good electrocardiographic signal at peak exercise, signal detection problems secondary to motion artifact may lead to superimposition and averaging of diastole and systole and underestimation of ventricular function. Although gating problems with exercise are observed in all active nuclear cardiology laboratories, there has been no systematic study of their frequency or influence on results. None of the studies reporting on exercise-induced asynergy in the absence of coronary artery disease has described repeat evaluation of these patients to exclude the possibility of technical artifact of a poorly gated study.

Also, normal differences in regional wall motion that are not apparent at rest may theoretically be perceived as new regional asynergy with exercise in the presence of either a gating artifact or hypokinesis that is equally distributed between all segments. Thus, the normally more contractile lateral wall may appear only normal during exercise, while apex and septum now appear hypokinetic. Therefore, the possibility of technical artifact must be considered in evaluating the significance of exercise-induced asynergy either in the clinically normal patient or in the patient with noncoronary myocardial disease.

Finally, the determination of regional asynergy by radionuclide ventriculography remains a subjective determination. Interobserver or intraobserver variability may occasionally account for false-positive readings. Overreading of wall motion abnormalities, as in overreading of any noninvasive test, will result in a higher sensitivity at the expense of a lower specificity.

Conclusions

Exercise-induced regional dysfunction appears to be nonspecific for coronary artery disease, and occurs both in "normal" populations and in persons with other cardiac diseases. The possibility of technical error or artifact must always be considered in any computer-dependent technique, but the frequency of this potential cause is unknown. In addition, differences in perceptual threshold for regional asynergy may also account for positive readings. Primary cardiomyopathic processes, abnormalities in vascular tone, and valvular heart disease must also be included as possible etiologies in the interpretation of exercise-induced asynergy. It is reasonable to postulate that regional population differences in age, prevalence of coronary artery disease, presence of valvular or myocardial disease, and referral bias may explain differences in specificity for exercise-induced asynergy obtained in different laboratories.

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Alcohol Withdrawal and Blood Pressure

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Abstract

Blood pressure elevation has been reported to be associated with alcoholism and alcohol use. This study assesses changes in blood pressure and risk factors associated with elevated pressures among chronic alcoholics admitted for withdrawal. Blood pressure measurements of 482 patients were analyzed. Both systolic and diastolic pressure decreased significantly from admission to discharge. At discharge, elevated pressure was associated with being black and male, and with a history of hypertension. Elevated pressure was not associated with age or obesity. Compared with U.S. population data, the young males in our study had increased prevalence and the older males had decreased prevalence. Elevated pressure is associated with alcohol withdrawal. The effect of alcohol should be considered in the office patient with elevated blood pressure.

Although a recent study on alcohol use and hypertension concludes, "Up to 30% of hypertension in affluent countries may prove to be attributable to the use of alcohol" (1), alcohol abuse and alcoholism are rarely included in differential diagnostic lists for secondary hypertension.

Blood pressure (BP) is reported to be influenced by alcohol intake and alcohol dependence (2–6). Klatsky reported an increase in BP among those reporting they had more than three drinks a day (7). A recent population study suggested a linear relationship between alcohol use and BP among men (8). Saunders et al reported an association between the level of BP elevation and the severity of withdrawal among alcoholics (9), and Mehta and Sereny reported a consistent drop in diastolic and systolic pressure during alcohol withdrawal (10).

The study we report here assessed BP changes and the risk factors associated with elevated pressures among a large number of chronic alcoholics admitted for alcohol withdrawal.

Methods

The potential study population included all patients admitted from October 20, 1979 through March 1, 1980 to the Beth Israel Medical Center Alcohol Treatment Program inpatient unit, a voluntary 50-bed service which utilizes a tapering dose of phenobarbital to withdraw patients from physical dependence on alcohol. The majority of patients are ambulatory by the second hospital day and are expected to participate in therapeutic acitivities. Patients with severe medical problems are transferred to another service.

Blood pressure was measured by mercury manometers calibrated prior to and during the study. All measurements were made by nurses specifically instructed in the need for consistent practice. Systolic pressure was recorded at the appearance of Korotkoff sounds and diastolic at their disappearance. Measurement was made on the right arm with the patient seated, at admission and thereafter each morning until discharge. The admission BP is the value obtained in the admitting area. Each patient was seen in individual rooms for a health review prior to admission to the hospital ward. Data were obtained from patient charts by trained abstractors.

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Results

Study Population. Of the 734 patients admitted to the hospital in the study period, 108 had a length of stay of less than three days and were excluded from the study. Of the 626 patients admitted to the study, charts of 482 (77%) had complete demographic and BP data and were used for analysis. A total of 144 patients were excluded from analysis because age, ethnicity, sex, or hypertension history was unknown, or because phenobarbital treatment was not discontinued at least one day before their last recorded BP. The mean admitting BP, last recorded BP, and age of the excluded population did not differ significantly from those of the population for data analysis. The sex and ethnicity distributions of the two groups were also not significantly different.

All patients included in analysis had stopped taking phenobarbital for at least 24 hr; 91% had stopped at least 48 hr prior to the last BP measurement. The initial dose of phenobarbital for patients requiring medication was generally 90 mg intramuscularly at admission, with a tapering schedule of oral medication thereafter. The majority (75%) of patients received 120 mg or less as the initial-day oral dose with rapid tapering to no phenobarbitol over 3 days. The study population was predominantly male (84%), and 64% were less than 45 years old. Twenty-nine percent of the group were white, 48% black, and 23% Hispanic. Mean number of days in the hospital prior to the last BP recording was 6.5.

Mean (±1 SD) admitting pressures were 143 (±20) systolic and 97 (±14) diastolic. Mean discharge pressures were 124 (±16) systolic and 81 (±12) diastolic. Admission and last systolic and diastolic BP were significantly different. Figure 1 demonstrates the reduction in mean diastolic pressure by sex for the three ethnic groups.

Prevalence of Elevated Blood Pressure. Patients were classified as having elevated BP if systolic pressure was equal to or greater than 140 mmHg or diastolic pressure was equal to or greater than 90 mmHg. Using this criterion, 78.2% of patients had elevated admitting BP. Age, sex, and ethnicity were not associated with elevated admitting BP. At the last BP measurement, 39.4% had elevated BP. The difference in prevalence is statistically significant ($\chi^2 = 10.96, p < 0.001$). At discharge there was a statistically significant relationship of BP elevation to sex and ethnicity, but not to age (Table 1). Black males had the highest prevalence of elevated BP.

A weak but significant association of discharge and admission BP status was found ($\chi^2 = 7.196, p < 0.025$). Of subjects with elevated discharge BP, 85% had elevated BP at admission; however, only 40% of subjects with elevated admission BP continued to have elevated BP after withdrawal. The 15% of alcoholics with normal admission but elevated discharge pressures included primarily individuals with borderline pressures on both admission and discharge, as well as a few with circulating diazepam on admission.

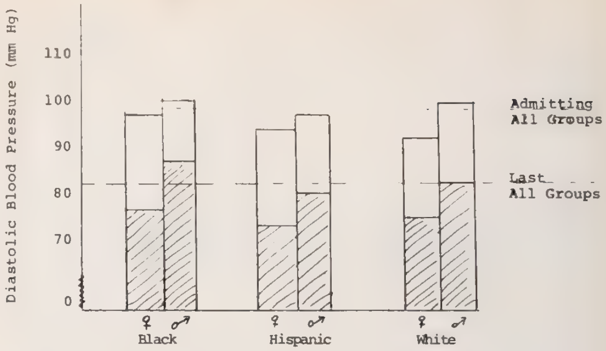

Fig. 1. Mean admitting and last recorded diastolic blood pressure by ethnicity and sex.

Associated Risk Factors. A history of hypertension or high BP was reported on admission by 188 patients (39%). A history of high BP was significantly but very weakly associated with admitting BP; the relationship with postwithdrawal BP elevation was stronger. Of patients with elevated BP at discharge, 53% had a positive history, compared to only 44% at admission.

Classification of severity of withdrawal was indirectly examined utilizing the phenobarbitol dosage schedule ordered by the clinicians. Those patients with two days or more on phenobarbitol

TABLE 1

Relationships of Sex and Ethnic Group to Last Recorded Blood Pressure

	Last blood pressure		
	Normal (*n*)	Elevated [*n* (%)]	Total (*n*)
Sex			
Male	238	169 (42)	407
Female	54	21 (28)	75
Total	292	190 (39.4)	482
χ^2 (1 df) = 4.3, $p < 0.03$			
Ethnic group			
Black	126	104 (45)	230
Hispanic	77	34 (31)	111
White	89	52 (37)	141
Total	292	190 (39.4)	482
χ^2 (2 df) = 7.209, $p < 0.03$			

TABLE 2

Relationship of Hypertension History and Blood Pressure Status

History	Admitting BP			Discharge BP		
	Normal	Elevated	Total	Normal	Elevated	Total
Negative	82	212	294	206	88	294
Positive	23	165	188	86	102	188
Total	105	377	482	292	190	482
		χ^2 (1 df) = 15.592, $p < 0.001$			χ^2 (1 df) = 27.4, $p < 0.001$	
		R = 1.217.			R = 1.813.	

were classified as moderate to severe withdrawal. Based on this classification, there is an association with admitting BP (Table 3); 67.2% of patients with mild withdrawal had elevated BP, whereas 85.7% of those with moderate/severe withdrawal had elevated BP. There is also an association with final BP; 32.2% of patients with mild withdrawal and 44.2% of patients with moderate/severe withdrawal had elevated final BP. In the groups defined by withdrawal severity, admitting and last BP are not associated.

Because obesity is associated with elevated blood pressure, Quetelet's index of obesity was used to assess the association of weight with BP among black and white men aged 25–74. An increased prevalence of elevated last BP was found with increasing overweight, but this association was not statistically significant (Table 4). A possible interrelationship of age, ethnicity, and obesity with BP was examined. Within ten-year age groups, the prevalence of elevated last BP increased with increasing Quetelet's index (not statistically significant) in both whites and blacks. When the obesity index was controlled, no association of age and last BP was found.

Comparison to General U.S. Population. Although the rate of elevated BP in the study population is markedly lower after withdrawal, the prevalence of elevated BP among the alcoholic patients at discharge may not be similar to a comparable group of nonalcoholic persons. Although no control group was available for study, we compared the age and sex-specific prevalence of elevated BP, by ethnic group, to that of the U.S. population. The younger males in our study had an increased prevalence of elevated last BP and the older males had a decreased prevalence relative to the U.S. population (Table 5). Although not statistically signficant, this pattern is consistent in both ethnic groups.

TABLE 3

Severity of Withdrawal and Blood Pressure Status

Withdrawal	Normal	Elevated	Total
Admitting BP			
Mild	64	131 (67.2%)	195
Mod/severe	41	246 (85.7%)	287
	105	377 (78.2%)	482
		χ^2 (1 df) = 22.33, $p < 0.001$	
Last BP			
Mild	132	63 (32.3%)	195
Mod/severe	160	127 (44.3%)	287
	292	190 (39.4%)	482
		χ^2 (1 df) = 6.44, $p < 0.01$	

Discussion

The findings presented here are consistent with those of other authors (10, 11) who reported that persons dependent on alcohol have elevated BP which decreases after withdrawal from alcohol dependence. The association of last BP with sex and ethnicity in our study, compared with no association for admitting BP, strengthens the secondary nature of elevated BP among alcoholics prior to withdrawal.

Whether BP is also elevated during active alcohol consumption and a rising or stable blood alcohol level is not addressed by this study. Available literature on this issue is inconclusive (12, 13). However, since alcoholic persons experience repeated cycles of relative withdrawal states whenever the blood alcohol level is falling or goes below a threshold, it seems reasonable to postulate that they have frequent elevations of BP secondary to withdrawal.

TABLE 4

Obesity and Last Blood Pressure

Quelelet's index*	Last blood pressure		
	Normal	Elevated	Total
≤3.5	115	86 (42.8)	201
3.5–3.9	34	37 (52.1)	71
≥4.0	12	17 (58.6)	29
Total	161	140 (46.5)	301

* ≥3.5 is considered obese.

TABLE 5
Prevalence of Elevated Blood Pressure after Withdrawal: Study Population and U.S. General Population (15) by Age and Ethnic Group (Male)*

	Black		White	
Age	Study % prev (*n*)	U.S. % prev	Study % prev (*n*)	U.S. % prev
18–24	50.0 (4)	15.2	40.0 (5)	19.6
25–34	45.1 (51)	35.4	51.2 (17)	27.6
35–44	48.7 (76)	60.6	32.4 (34)	35.9
45–54	48.1 (54)	53.8	45.5 (26)	53.3
55–64	71.4 (7)	68	45.0 (20)	58.0
65–74	0 (1)	78.7	33.3 (6)	64.0
All ages	48.2 (193)	45.4	40.0 (108)	38.9

* Black $\chi^2 = 4.03$, 5 df, NS. White $\chi^2 = 7.76$, 5 df, NS.

The association of BP at admission with severity of withdrawal as measured by the phenobarbital schedule is consistent with the findings of Saunders (14). However, the severity classification was also associated with elevated last BP, although there was no association within the severity groups between admitting and last BP. The indirect nature of the severity classification may have confounded this examination.

The question of whether alcoholism may lead to persistent elevation of BP after alcohol withdrawal is not definitely answered by this study, because there is no control group or long-term follow-up. The comparison to the U.S. population, however, shows a clear trend. It can be speculated that the pattern of increased prevalence among the young and decreased prevalence among older persons in our study could be due to a permanent hypertensive change caused by chronic alcohol use which is not seen in the older group because the hypertensives have been selectively eliminated from the age cohort by hypertension-related death.

Summary

Various reports estimate that 5%–10% of the U.S. population and perhaps even higher proportions of persons seen in a physician's office have the disease alcoholism or significant alcoholism problems. The impact of such prevalence on the diagnosis and treatment of hypertension is substantial. This study suggests that the alcohol abuser seen in the physician's office with a sustained elevated BP should not be automatically labeled as having essential hypertension. In addition, if secondary hypertension is to be assessed in a patient with high BP, alcoholism should be among the diseases investigated. The initial treatment for alcohol-induced hypertension is the same as for any other secondary hypertension: treatment of the underlying cause—in this case, alcoholism. The hypertensive alcoholic person who is unresponsive to alcoholism treatment should be treated like any other hypertensive.

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The Histiocyte and the Lung: A Radiologic Approach to Classification of Histiocytic Pneumonopathies

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The role of the lymphocyte and of the eosinophil in the lung is well known; that of the histiocyte is not. This report presents a classification of pulmonary diseases having histiocytic predominance.

Definition of the Histiocyte. The histiocyte is a ubiquitous cell belonging to the reticuloendothelial system and is derived from the undifferentiated pluripotential mesenchymal cell (Fig. 1) (1, 2). It is a differentiated cell with specific functions, of which phagocytosis is the most important. For this reason, a histiocyte is also known as a fixed phagocytic cell (3) or tissue monocyte (2).

Histiocytes can adopt a variety of forms including that of foam cells, giant cells, spindle cells, and fibroblasts (Fig. 1). The alveolar macrophage is an example of a modified histiocyte. The histiocytic cell series is a continuum, starting from the progenitor in the bone marrow (undifferentiated pluripotential cell), progressing to the intermediate blood monocyte, and finally terminating as a tissue macrophage or histiocyte (4).

Functions. When stimulated in the loose connective tissues, the histiocyte is capable of fibroblastic transformation (Figs. 1, 2). In the lung, the histiocyte is a fusiform cell capable of assuming a rounded shape when inflammation occurs. When blood monocytes migrate from the vascular circulation to the tissues, they become indistinguishable from the histiocyte (Fig. 1) (5). Also, the histiocyte may become an epithelial-like cell. When grouped, these cells form granulomas. If fusion or amitotic division occurs, Langhans, foreign body, and Touton giant cells result.

The histiocyte not only plays an important role in the maintenance of the local defense in the lung against bacterial and protozoal infections, but it also ingests extravasated erythrocytes, cellular debris, inert foreign matter, and both exogenous and endogenous lipids. When filled with lipid, it is termed "xanthoma" or "foam cell."

Classification of Histiocytic Pneumonopathies

The classification of histiocytic diseases has not been adequately determined, but the histiocyte is histologically the predominant cell in the lesion in several entities. Not all histiocytic diseases involve the respiratory system but in many of them, the lung is either primarily affected or is among the systemic manifestations of the disease. Pulmonary histiocytic diseases can be classified as metabolic, inflammatory, reactive, or neoplastic (Table).

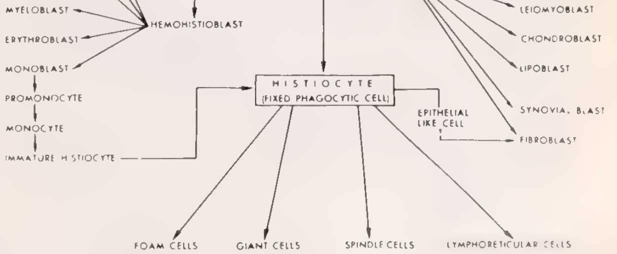
PLASMABLAST
LYMPHOBLAST
MEGAKARIOBLAST
MYELOBLAST
ERYTHROBLAST
MONOBLAST
PROMONOCYTE
MONOCYTE
IMMATURE HISTIOCYTE
HEMOHISTIOBLAST
UNDIFFERENTIATED MESENCHYMAL (RETICULAR) CELL
HISTIOCYTE (FIXED PHAGOCYTIC CELL)
EPITHELIAL LIKE CELL
OSTEOBLAST
RHABDOMYOBLAST
LEIOMYOBLAST
CHONDROBLAST
LIPOBLAST
SYNOVIAL BLAST
FIBROBLAST
FOAM CELLS
GIANT CELLS
SPINDLE CELLS
LYMPHORETICULAR CELLS

Fig. 1. Relationship of the histiocyte to other cell types of mesenchymal origin. Adapted from Kern (1) and Pear (2).

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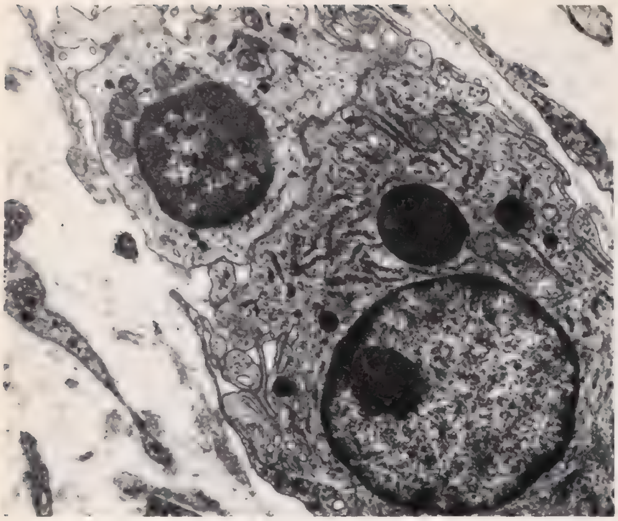


Fig. 2. Electron microscopy section illustrating capability of fibroblastic transformation in histiocytes. **Top left cell:** Histiocyte in phase of transformation into fibroblast. Note cytoplasmic extensions (fibroblastic feature) and granular cytoplasm (histiocytic feature). **Bottom right cell:** Histiocyte already transformed into fibroblast with some histiocytic characteristics of lysosomes (Type I) or heterolysosomes. *(Courtesy of V. Pardo, M.D., Department of Pathology, Veterans Administration Hospital, Miami, Florida)*

Metabolic Lesions

Among its multiple attributes, the histiocyte is capable of phagocytosis. In metabolic processes where there is overproduction of lipid substances, the histiocyte has the ability to accumulate this material in its cytoplasm, as in xanthoma diabeticorum, xanthelasma, Gaucher's disease, and Niemann-Pick disease (6).

Inflammatory Lesions

The pulmonary histiocyte can proliferate in infections and can adopt an epithelial-like appearance, forming pulmonary granulomas with multinucleated cells (7). Classical examples are tuberculosis, sarcoidosis, and some deep mycoses. Included in these lesions is one of the noninfective pneumonias, lipid interstitial pneumonitis (8), which can be either exogenous or endogenous.

TABLE

Spectrum of Pulmonary Lesions Containing Histiocytes (Fixed Macrophage or Phagocytic Cell)

Metabolic
Niemann-Pick
Inflammatory
Endogenous lipid pneumonia
Exogenous lipid pneumonia
Specific granulomas: TB, Boeck's sarcoid
Reactive (post-inflammatory pseudotumors)
Histiocytoma
Plasma cell granuloma
Xanthogranuloma
Fibrohistiocytoma
Sclerosing hemangioma

Neoplastic
Histiocytoma
Plasma cell granuloma
Xanthogranuloma
Fibrohistiocytoma, benign & malignant
Sclerosing hemangioma
Eosinophilic granuloma
Hand-Schüller-Christian
Letterer-Siwe
Systemic malignant histiocytosis
Reticulum (histiocytic) cell sarcoma or lymphoma

Adapted from Pear (2).

Endogenous. The endogenous type is usually the result of an inflammatory process in which tissue necrosis plays an important role with liberation of the lipid, which is then phagocytized by the pulmonary histiocytes.

Lipid pneumonia was described originally by Waddell in 1949 (9). On the basis of pH changes which alter the colloidal state of proteins and lipids, he determined that the etiology of this process was related to the deposition of natural fat, phospholipids, and cholesterol in areas of inflammation. Perhaps of greater importance is the presence of obstructive lesions at the level of the respiratory bronchioles, as described by Gosink et al (10).

The radiologic appearance is usually that of a lung mass, found serendipitously in an asymptomatic patient, and associated with pulmonary consolidation or atelectasis. Because of these findings, the differential diagnosis of a lung neoplasm is usually entertained. If there is evidence of an air bronchogram within the infiltrate, and it appears to have negative absorption coefficient values on CT, a bronchogenic neoplasm must be ruled out in most cases (Fig. 3).

Gross examination of these lesions at an early stage reveals a bright yellow area of firm consolidation which in later stages, when intraalveolar organization with fibrosis occurs, is transformed into a grayish lesion. Microscopically, there are foam-filled macrophages containing cholesterol crystals and cholesterol esters. These lipid substances tend to be terminally dispersed in the cytoplasm of histiocytes.

Exogenous. In the exogenous type, aspiration of oily nasal drops, cod liver oil, or mineral oil reaches the lung. The only etiologic and clinical relationship between the endogenous and exogenous types is that in each case histiocytes play the same role. Microscopically, exogenous and endogenous lipid pneumonia are identical. Radiologically, one or several areas of consolidation in the dependent zones of the lung may be observed. The lymphatics of the lung are often prominent, suggesting lymphatic permeation by tumor and leading to a differential diagnosis of lung carcinoma. Another differential point between endogenous and exogenous lipid pneumonia is the gravitational distribution of the latter.

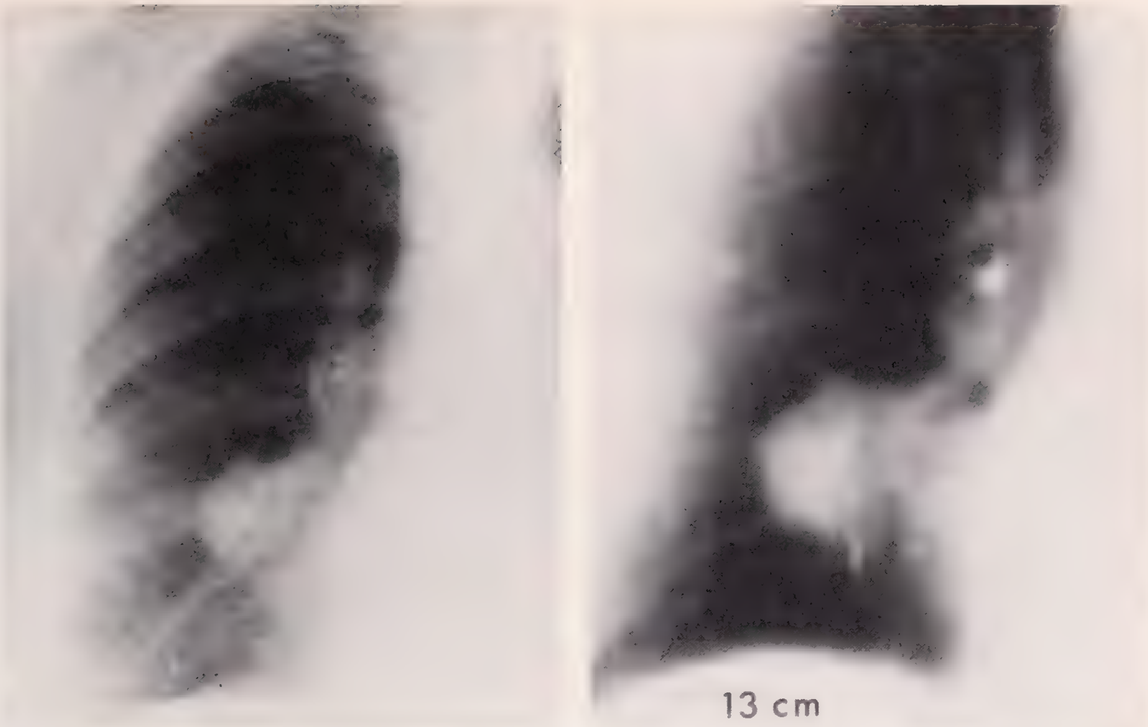
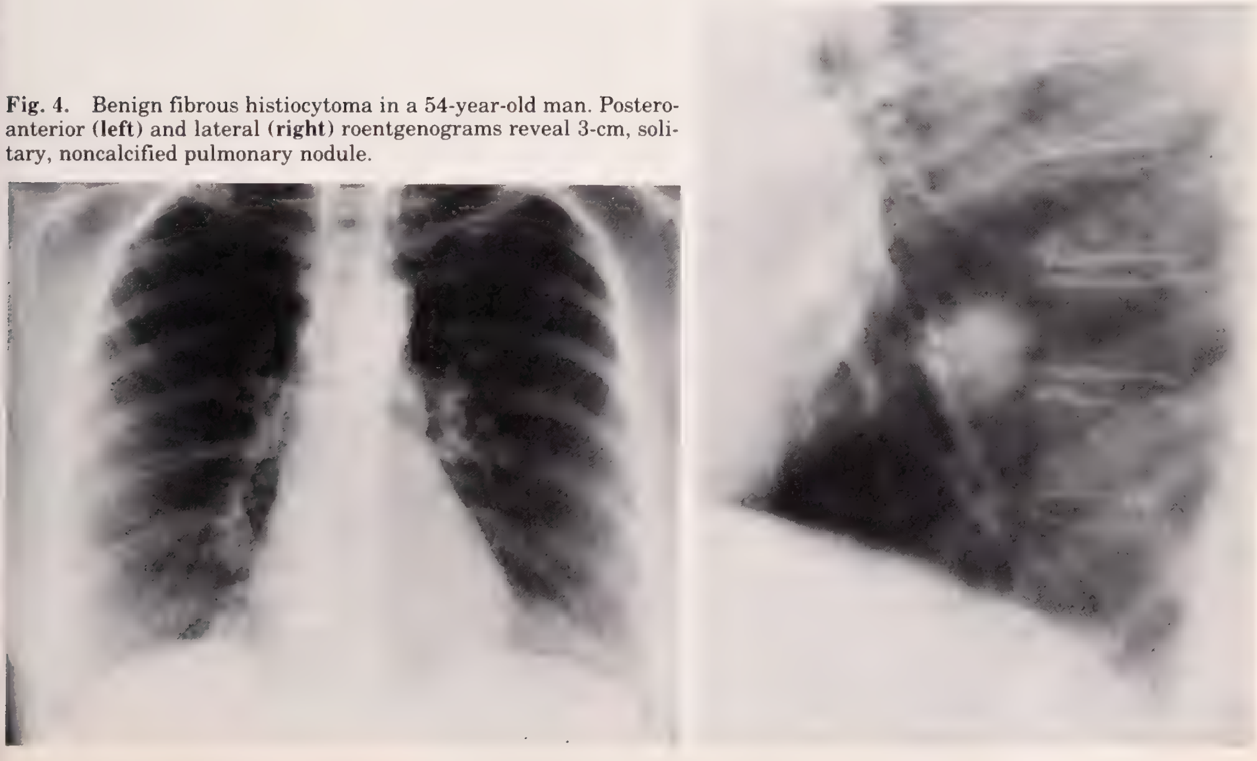


Fig. 3. Endogenous lipid pneumonia in a 52-year-old woman with a 4-week history of hemoptysis. **Left** Consolidation of right middle lobe is noted in frontal and lateral chest roentgenograms. **Right** Tomogram of same patient. Middle lobe mass appears lobulated and an air bronchogram is noted.

Fig. 4. Benign fibrous histiocytoma in a 54-year-old man. Posteroanterior (**left**) and lateral (**right**) roentgenograms reveal 3-cm, solitary, noncalcified pulmonary nodule.



Neoplastic Lesions

Benign Pulmonary Fibrous Histiocytoma and Related Lesions. Advances in thoracic surgical techniques during the 1950s have brought an emphasis on resection of pulmonary nodules. Discovery of histiocytic tumors among these nodules led to a new classification system, first elucidated in publication by Scott et al (11).

Umiker and Iverson in 1954 reported four cases of postinflammatory pulmonary tumors, setting a baseline for recognition of this lesion. They observed and described the astonishing morphologic resemblance of these pulmonary masses to certain cutaneous neoplasms, such as xanthoma, fibroma, plasma-cell tumors and vascular tumors (12). The term *postinflammatory pseudotumor* was employed because of the abundant inflammatory component, its benign behavior, and the history in all cases of an upper or lower respiratory tract infection. Liebow and Hubbell in 1956 described the existence of pulmonary tumors histiologically similar to *cutaneous* sclerosing hemangiomas (13) which had been reported by Lister in 1938 (14). This pulmonary neoplasm revealed a proliferation of blood vessels, presence of hemorrhages, abundant histiocytic infiltration, and focal fibrosis. The same authors mentioned the possibility that these pulmonary sclerosing hemangiomas might be *xanthomas* or *histiocytomas* due to the large amount of lipid material in the absence of hypercholesterolemia.

In 1962, Titus et al (15) published an extensive revision of the literature and a list of 25 cases of *benign fibrous histiocytomas* of the lung and related lesions. For these authors, the spectrum of histiocytic lesions represented either the end-stage of diverse etiologic processes, or lesions that had the same etiology but were found at different stages of development.

To avoid discrepancies in the terminology, Stout and Lattes chose to encompass all these benign pulmonary histiocytic lesions under the term *benign fibrous histiocytoma,* and this is the term that appears in the 1967 Armed Forces Institute of Pathology *Atlas of Tumor Pathology* (16). The term includes xanthoma, xanthofibroma, plasma-cell granuloma, histiocytoma, sclerosing hemangioma, and fibrous xanthoma.

Benign fibrous histiocytoma can affect parts of the respiratory tract other than the lung: trachea (17, 18), bronchi (19), paranasal sinuses. Its incidence is slightly higher in women than in men and it is more prevalent in the third and fourth decades of life, even though cases have been reported in all age groups.

The benign pulmonary form is the most frequent tumor mass in the lung in patients under the age of 16 (20). Given the excellent prognosis, differentiation from other malignant pulmonary neoplasms is vital. Clinically, there are no characteristic physical findings. Less than 40% of cases have symptoms related to the endobronchial growth of the tumor, such as cough, sporadic hemoptysis, chest pain, and dyspnea (21).

Radiographically, benign fibrous histiocytoma of the lung appears as a well-circumscribed, nearly homogeneous, rounded mass. It can be located anywhere in the pulmonary parenchyma, but seems to prefer a peripheral location and the lower lobes (Fig. 4). The growth rate, determined by serial radiographs, is variable. It may remain stable for years, or may grow rapidly in a few months. Rapid growth has been described following lung infections (6).

Certain macroscopic features are characteristic. The tumors are sharply circumscribed and have a defined pseudocapsule. A yellowish-white to deep-yellow color is suggestive of their xanthomatous component (22). Their size can vary, from 2 cm to destruction of an entire lung. They can have pseudopod-like extensions projecting into the bronchi and thereby causing obstruction, or they can invade the mediastinum or pleura, making surgical resection difficult. In early stages, the consistency of these lesions is soft and friable. Gradually they may become fibrotic, and finally calcification and ossification may occur. In chronic cases, they can cavitate. When the vascular component predominates (as in sclerosing hemangioma) a reddish color, resulting from hemorrhages (13), is seen.

Histologically, these lesions are polymorphic, with the presence of plasma cells, lymphocytes, lipid-laden histiocytes, fibroblasts, and a variable vascular component (23). The appearance varies with composition, but the common denominator is the xanthomatous component (24). The histiocytes are large, of uniform size, and may show evidence of mitosis. They vary greatly, from spindle-shaped cells, to foam cells, to multinucleated giant cells. The cells are usually arranged in lobules whose periphery consists of spindle cells that acquire more cytoplasm as they approach the center, where they appear vacuolated and contain more lipid. The presence of lipid—intracellularly (in the form of foam cells) and extracellularly—is the most outstanding histologic feature.

Because neither the radiologic nor the macroscopic appearance is uniquely characteristic, the only diagnosis that can be made is that of a nodular lesion.

The differential diagnosis radiologically of benign pulmonary fibrous histiocytoma includes granulomas, hamartomas, and bronchial adenoma. Other, more unusual entities include leiomyomas, papillomas, fibromas, lipomas, and endometriosis (25). The presence of calcification, although not pathognomonic, suggests the possibility of granulomas or hamartomas. Lipomas, bronchial adenomas, and leiomyomas tend to obstruct the bronchial airway more often than the fibrous histiocytoma. Lung papillomas are commonly multiple and associated with laryngeal papillomatosis. In endometriosis, pneumothorax and hemoptysis may occur during menstruation. In differentiating between the benign fibrous histiocytoma and its malignant counterpart, the absence of hilar adenopathies or pleural effusions favors the former.

The prognosis is excellent in benign fibrous histiocytoma. Local recurrence has not been reported after wide local resection including a rim of normal lung.

Malignant Pulmonary Fibrous Histiocytoma. In 1979, Kern et al first described malignant fibrous histiocytoma of the lung (1). This lesion is analogous to malignant fibrous histiocytoma of the soft tissues and is the malignant counterpart of benign fibrous histiocytoma of the lung (26). The ultrastructural appearance of this lesion reveals that it originated from undifferentiated mesenchymal cells, and is therefore a mesenchymal sarcoma (27). Although malignant fibrous histiocytoma is the most frequent sarcoma of soft tissues in adults (28), surprisingly, only a few cases have been reported in the lung; the confusion in classification of histiocytic tumors may be responsible (20).

The clinical signs are completely nonspecific, and may in fact be absent. Symptoms of cough, chest pain, and low-grade fever have usually been the reason for the roentgenographic examination of the chest.

The discovery of these lesions has usually been serendipitous. The radiologic appearance is that of a nodular lesion in two different patterns: solitary pulmonary nodule (4, 24) of variable size (2–1 cm), well defined and lobulated, and not containing calcium. (Fig. 5); or nonspecific chronic infiltrate (22), frequently involving the pleura and/or the chest wall musculature (Fig. 6).

Macroscopically it is a well-circumscribed, firm mass with a yellow-white tinge. The histopathologic features are the same as in malignant fibrous histiocytoma of the soft tissues, including storiform pattern, numerous mitotic figures, and pleomorphism (29).

The prognosis is poor and the survival rate for five years is less than 50% (1). Surgical excision is the recommended treatment. Early metastases have been described in two of the six cases studied and local recurrence is often seen.

Histiocytosis X: The group of related lesions identified as histiocytosis X are considered different forms of the same disorder, having many features consistent with a neoplastic condition (30). The disorder may affect the lung as one of many organs involved. Here again, the predominant cell type is the histiocyte.

Letterer-Siwe disease. Letterer-Siwe disease, or acute histiocytic reticulosis, is a fatal condition occurring usually in infants and young children that pursues a rapid course. The clinical picture includes a scaly, papular rash primarily in the scalp; enlargement of the spleen, liver, and lymph nodes; and radiologic lesions in the bones. The pulmonary involvement begins in the upper lobes and later may progress into a diffuse reticulonodular pattern.

Radiologically, a rapidly progressive reticular pattern is noted, ending with complete replacement of the pulmonary parenchyma by pneumatoceles. Macroscopically, the lungs have a honeycomb appearance in the later stages, with cysts measuring less than 1 cm in diameter and having walls composed mostly of grayish-white tumor tissue. Microscopically, this disease is characterized by the presence of histiocytes (appearance usually foamy) and variable numbers of eosinophils and giant cells. The amount of reticulum fibers is proportional to the chronic evolution of the lesion. The pathogenesis of the cystic changes is controversial but bronchial weakening is believed to be due to diffuse pleural infiltration causing a valve-like mechanism in the alveoli, resulting in overdilatation.

Hand-Schüller-Christian disease. This entity was first described as a syndrome with xanthomatous deposits in bone, diabetes insipidus, and exophthalmos. Its course is slowly progressive; although the diagnosis is made during infancy, the patient usually reaches adulthood. Clinical symptoms include dyspnea and cough preceding the radiologic changes in the lung considerably (31).

Radiologically there are interstitial changes resembling interstitial pneumonia and progres-

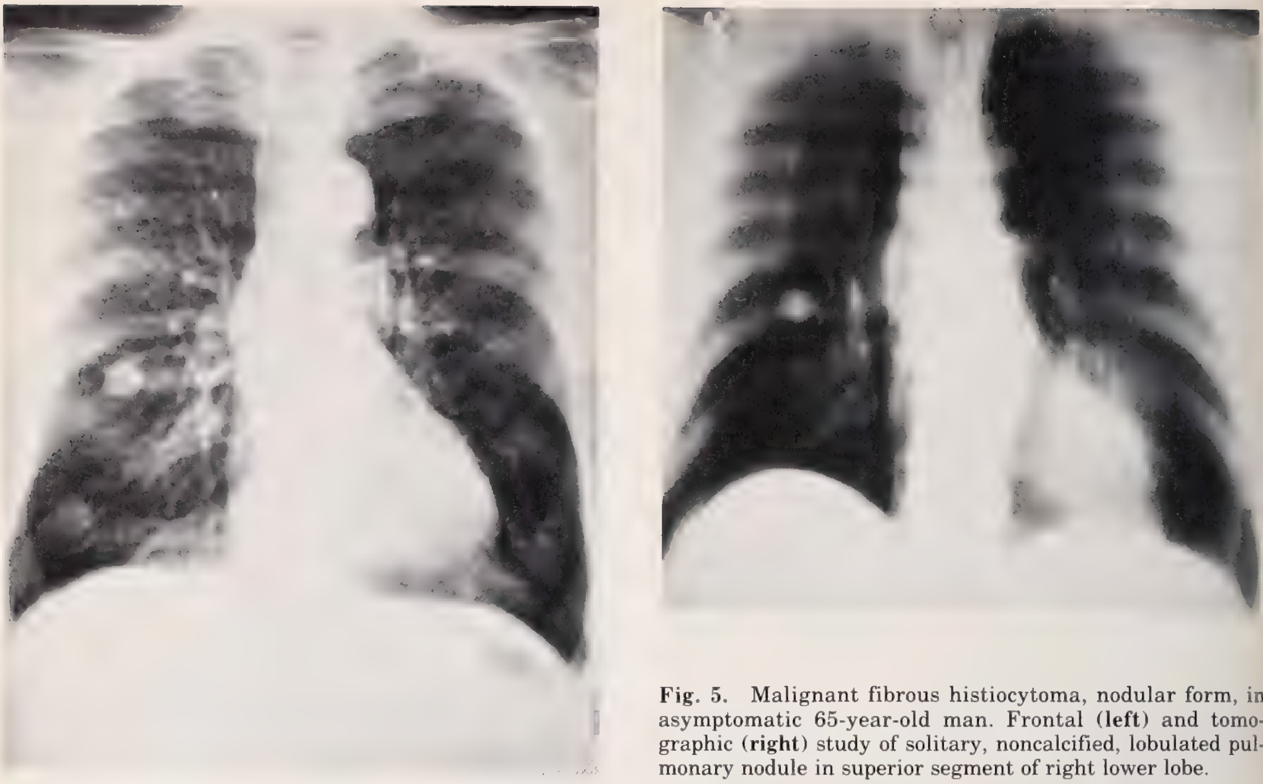


Fig. 5. Malignant fibrous histiocytoma, nodular form, in asymptomatic 65-year-old man. Frontal (**left**) and tomographic (**right**) study of solitary, noncalcified, lobulated pulmonary nodule in superior segment of right lower lobe.

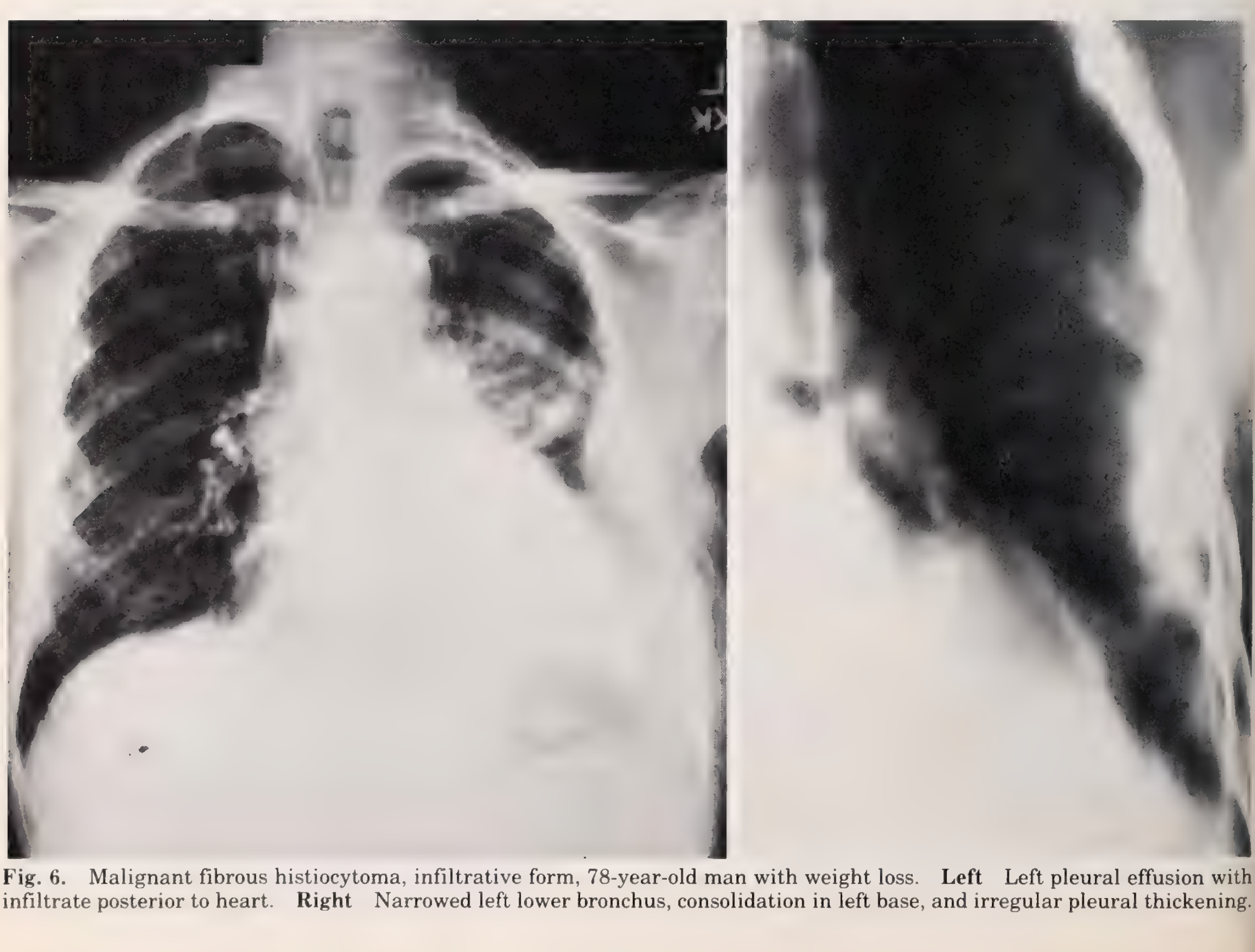


Fig. 6. Malignant fibrous histiocytoma, infiltrative form, 78-year-old man with weight loss. **Left** Left pleural effusion with infiltrate posterior to heart. **Right** Narrowed left lower bronchus, consolidation in left base, and irregular pleural thickening.

sion to a honeycomb pattern of peripheral predominance.

Macroscopic appearance is that of diffuse pulmonary fibrosis containing small deposits of orange-yellow lipid (cholesterol esters) and many small cysts. Microscopically, there is infiltration by multinucleated histiocytic cells and fibroblasts.

Perivascular involvement can cause infiltration of the adventitia, narrowing of the arteries, and, finally, pulmonary hypertension. Histiocytes are laden with cholesterol, probably the result of obstructive pneumonitis and hemorrhage.

The prognosis is better than that of Letterer-Siwe disease; mortality rate in five years is 20%–30%. The treatment does not include radiation therapy, but steroids and/or vinblastine.

Eosinophilic granuloma. This is commoner than Letterer-Siwe disease, has a better prognosis, and is more commonly found after puberty. The usual clinical symptoms include irritating cough; weight loss; and single or recurrent "spontaneous" pneumothorax. Pulmonary eosinophilic granuloma is the most common cause of spontaneous pneumothorax among youngsters.

The three entities that comprise histiocytosis X are radiologically similar, varying only in intensity and rapidity of evolution.

The pulmonary involvement by eosinophilic granuloma is diffuse and bilaterally symmetric, selectively affecting the upper lung fields. In the primary stages, nodular lesions less than 5 mm in diameter can be detected. Subsequently, a reticulonodular pattern is demonstrable (Fig. 7).

In the final stage of eosinophilic granuloma there is a classical reticular pattern with cystic lesions measuring up to 1 cm in diameter giving the typical honeycomb appearance. The hilar nodes are rarely involved in the adult although they can be in children. The concomitant finding of both pneumothorax and osseous involvement is frequent.

Macroscopically, four forms can be identified: firm grayish pulmonary masses with indistinct edges; diffuse miliary infiltrates; pleural plaque; honeycomb lung. Microscopically, the appearance is that of a granuloma with a predominance of large histiocytic cells. Also present is a variable number of eosinophils, lymphocytes, and plasma cells. In the early stages, hemorrhage and necrosis can be found. With the progression of the pulmonary lesions, vital and total air capacity is reduced, with an increase in residual air volume.

Radiation therapy is not advisable due to the fibrotic tendency of the disease, but steroid therapy may produce marked improvement if fibrosis has not caused severe lung destruction.

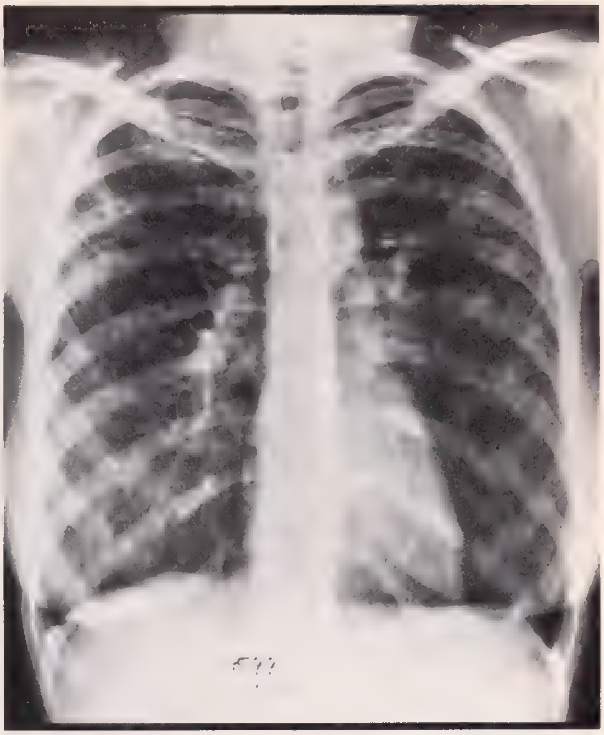


Fig. 7. Eosinophilic granuloma in 29-year-old man with shortness of breath on exertion and cough with whitish sputum of several years' duration. Fine, bright, symmetrically diffuse reticular pattern.

Pulmonary lymphomas. Reticulum (histiocytic) cell sarcoma is a very rare pulmonary neoplasm, comprising 0.5% of all primary pulmonary malignancies. Only 7% of all histiocytic lymphomas are considered pulmonary in origin (6). Its behavior tends to be malignant and is based more on the stage of the disease than on its histology. Involvement of the hilar lymph nodes implies a reduction in five-year survival. Its peak incidence is in the fourth decade of life, females being slightly more affected than males. It has a predilection for the upper lobes.

Radiologically, it usually appears as an asymptomatic solitary pulmonary mass with mediastinal lymph node enlargement. Macroscopic lymphomas of the lung present as a whitish mass varying from a few centimeters in size to an entire lobe replacement, having a soft fish-flesh consistency and ill-defined edges. Microscopically, these tumors are composed of histiocytes with characteristics of malignancy and invasion. The prognosis is generally considered poor. Chemotherapy is used with good results, even in patients in advanced stages.

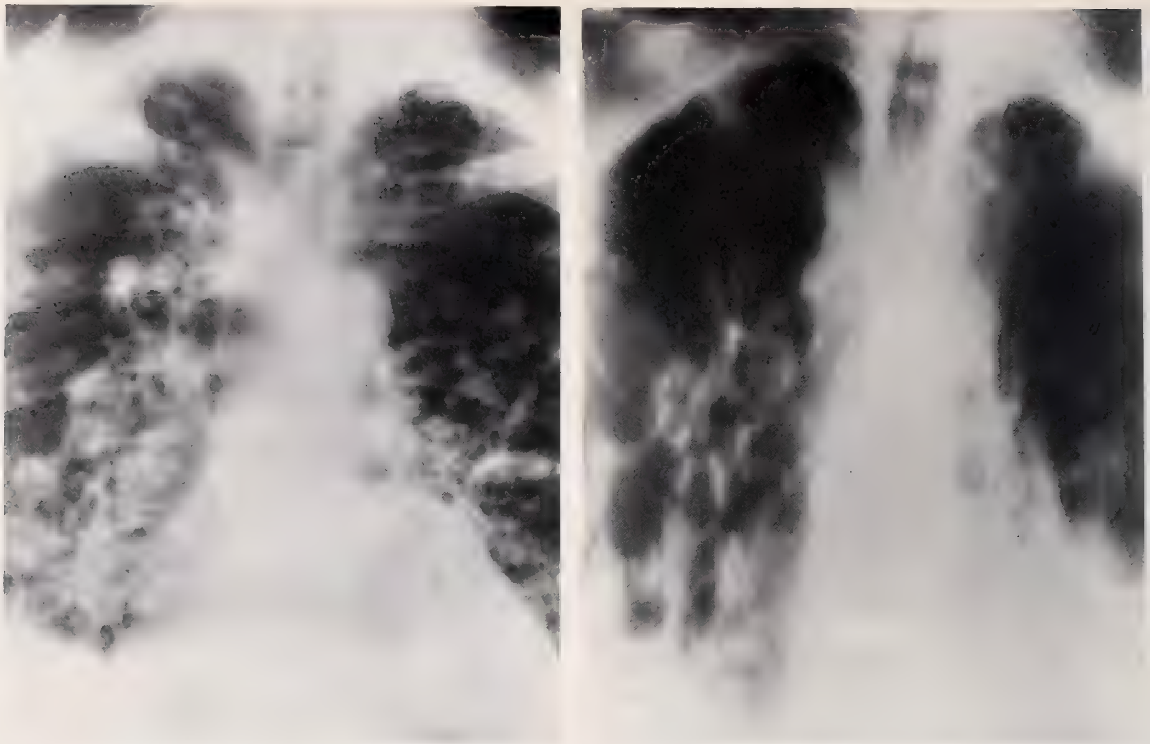


Fig. 8. Systemic malignant histiocytosis in 67-year-old man admitted because of cough, hemoptysis, and fever. **Left** Evidence of well-circumscribed rounded nodules bilaterally. **Right** In tomogram, nodular densities are more apparent; note right hilar adenopathy and tenting of right hemidiaphragm.

Systemic malignant histiocytosis. This entity was first reported by Scott and Robb-Smith in 1939, and since then over 100 cases have been described. It is characterized by a systemic, progressive, invasive proliferation of morphologically atypical histiocytes (32). The peak incidence is in the fourth decade of life, with a 2:1 male:female ratio (33). The major clinical findings are fever, generalized lymphadenopathy, hepatomegaly, and splenomegaly. Other symptoms include weight loss, chest pain, and skin rash. There is evidence of bilateral well-circumscribed rounded nodules and hilar lymph node involvement is frequent. Pulmonary parenchymal lesions progress rapidly with coalescence of the nodules (Fig. 8).

The macroscopic appearance is that of involvement of almost every organ, but principally lymph nodes, liver, spleen, bone marrow, kidney, and lung. In gross appearance, the lung has multiple diffuse, bilateral tumor nodules. The cut section of these nodules reveals a friable, necrotic dark-grayish material. The involved organs have diffuse infiltration by atypical histiocytes with variable cytologic atypia, irregular nuclei, and abundant mitosis. Diagnosis is usually by lymph node or bone marrow biopsy, which reveals infiltration by atypical histiocytes. Microscopically, the differential diagnosis should include histiocytic lymphoma, Hodgkin's disease, histiocytosis, undifferentiated malignant neoplasms, and viral lymphadenitis. The prognosis is very poor; a median survival is six months. Chemotherapy combined with radiation therapy is the treatment of choice but has little success.

Summary

The traditionally overlooked histiocyte is a principal component of a wide gamut of entities ranging from specific granulomas and histiocytic lymphomas to histiocytosis X. The discovery of the fibroblastic transformation (observed by electron microscopy) of the tissular histiocyte (34) has stimulated a reclassification of the histiocytic neoplasms, encompassing under the term "fibrous histiocytoma" the whole spectrum of pulmonary neoplasms with histiocytic component. In light of this new classification, the benign fibrous histiocytoma is now the primary pulmonary neoplasm most frequently found in persons under the age of 16 years (20). Primary malignant fibrous histiocytoma presents as two different patterns: a solitary nodule, or a chronic infiltrate with pleural extension and muscle wall invasion.

The radiologic findings in benign and malignant fibrous histiocytomas are nonspecific. The only definitive diagnostic method is study of histologic features. In the differential diagnosis of a solitary pulmonary nodule, fibrous histiocytoma should be included.

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Phenazopyridine (Pyridium) Poisoning: Possible Toxicity of Methylene Blue Administration in Renal Failure

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Abstract

A patient is described in whom the full spectrum of toxicities to phenazopyridine (Pyridium) developed. This was manifested by methemoglobinemia and cyanosis, acute renal failure, yellow skin and urine pigmentation, and a hemoytic anemia occurring after therapy with methylene blue (1 mg/kg). The development of hemolysis following methylene-blue-mediated correction of the methemoglobinemia raises the possibility of an erythrocytotoxic effect of methylene blue resulting from an impaired renal clearance of this compound. We suggest that guidelines for the administration of methylene blue in patients with renal failure be amended so that treatment be withheld or the dosage be reduced in patients with mild methemoglobinemia and cyanosis.

Phenazopyridine hydrochloride (Pyridium) is a popular urinary analgesic. Described toxic effects include methemoglobinemia (1–8), hemolysis (3–5, 7, 9–11), acute renal failure (7, 8, 12–14), and skin pigmentation (4, 8, 9, 12–14). Most of these reactions occur in patients with impaired creatinine clearance (1, 3, 9, 10, 13) or when a higher than recommended dosage of phenazopyridine (10 mg/kg/dy) is used (1–8, 12, 13). We report a patient who illustrates the full spectrum of toxic effects of phenazopyridine poisoning, possibly complicated by methylene blue administration.

Case Report. A 46-year-old man with a history of schizophrenia and polypharmacy abuse was admitted with a chief complaint of "yellow skin." Over the week prior to his admission he self-administered 30–40 tablets of phenazopyridine daily (100 mg/tablet) for symptoms of urinary hesitancy, frequency, and nocturia. On physical examination the positive findings were limited to deep yellow pigmentation of the sclerae, mucous membranes, and skin as well as prominent cyanosis of the lips, malar eminences, and ears. The blood pressure was 120/70. The blood urea nitrogen was 84 mg/dl and the serum creatinine 9.2 mg/dl (documented to be 1.1 mg/dl two years earlier). Serum electrolyte measurements were sodium, 134 mEq/l; potassium, 4.5 mEq/l; chloride, 97 mEq/l; and bicarbonate, 18 mEq/l. The hemoglobin was 13.9 g/dl, hematocrit 44%, and white blood cell count 16×10^3 with 83 neutrophils, 9 bands, 3 lymphocytes, 4 monocytes, and 1 atypical lymphocyte. The urine was deep orange-yellow, with a specific gravity of 1.012. It tested negative for protein or blood, and contained 0-2 white cells (no eosinophils) and occasional granular casts/high power field. The shake test results appeared grossly positive. Total serum bilirubin, however, was 0.3 mg%, and hepatic transaminases and serum proteins were normal. Arterial pH was 7.33, P_{CO_2} 22 mm Hg, and P_{O_2} 98 mm Hg. The methemoglobin level was 26.1% (normal nonsmoker 0%–3%).

For the first eighteen hours after admission, the patient was noted to be oliguric (15 cc/hr). In an attempt to induce diuresis he was treated with 400 mg of furosemide administered intrave-

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nously over a four-hour period and one oral dose of 10 mg metolazone, as well as 100 mg hydrocortisone sodium succinate every six hours. One dose of 70 mg (1 mg/kg) of methylene blue was administered intravenously to correct the methemoglobinemia. The patient's urine output increased to 200–300 cc/hr and over the next ten days the creatinine decreased to 1.8 mg/dl. The methemoglobin level decreased from 10% eight hours after methylene blue to 3% ten days later, while skin and urine pigment gradually returned to normal. On day seven the patient was asymptomatic with a hemoglobin of 9.2 g/dl, hematocrit of 27%, uncorrected reticulocyte count of 5%, lactate dehydrogenase of 437 mu/ml (normal 90–200), and negative stool guaiac and direct Coombs test. The patient was discharged with a daily oral folate supplement for his hemolytic episode. Two-month follow-up showed him to be normally pigmented, with creatinine of 1.0 mg/dl, hemoglobin of 14.2 g/dl, reticulocyte count of 1.0%, normal glucose-6-phosphate dehydrogenase, and a methemoglobin level of 0.3%.

Comments. The patient reported here manifested the entire spectrum of toxic effects of phenazopyridine poisoning. Phenazopyridine-induced methemoglobinemia (1–8) and hemolysis (3–5, 7, 9–11) have been described over the last several decades. Toxic levels of this urinary analgesic induce heme oxidation to methemoglobin at a rate that overwhelms the homeostatic capacity of red cells to maintain hemoglobin in the reduced state (15–18). At concentrations of 10%–25%, as in our patient, symptoms from methemoglobin are limited to cyanosis (15–18). Dyspnea, fatigue, and headache appear at intermediate levels; stupor and death occur with 60%–70% methemoglobin (15). In addition, there is a frequent parallel association between methemoglobin formation and Heinz-body hemolysis (16). More recently, phenazopyridine has been noted to cause a diminution in creatinine clearance (7, 8, 12–14), with biopsy-proven acute tubular necrosis in humans (12) as well as in dogs (19). This patient received three doses of hydrocortisone because of the initial consideration of an allergic interstitial nephritis as the cause of his oliguria. The yellow pigmentation is due to deposition of the azo-dye in the skin and sclerae and occurs primarily in patients with impaired renal function (4, 8, 9, 12–14). An erroneous diagnosis of hepatitis in our patient was entertained because of the yellow sclerae and the positive urine shake test and, in fact, a dose-independent hypersensitivity hepatitis has been described with phenazopyridine (20–22).

Methylene blue (1–2 mg/kg) is considered the treatment of choice for patients who are symptomatic from the methemoglobinemia or those in whom rapid increases in the oxidized pigment occur (15). This is based on the reduction of methylene blue in vivo to leukomethylene blue, which in turn reduces methemoglobin nonenzymatically. The drug is considered safe and effective at 1 mg/kg in adults and repeat administration is advised if cyanosis does not clear (18). However, higher doses (greater than 10 mg/kg) of methylene blue in cats (23) and human infants (24) has itself paradoxically been associated with Heinz body production and hemolysis. In addition, methylene blue is eliminated to a large extent by the kidneys (25–27). The temporal dissociation of methemoglobin normalization and subsequent hemolysis in our patient may certainly be due to an irreversible effect on the red cell set in motion by phenazopyridine or methemoglobin and manifesting itself days after measured oxidized hemoglobin returned to normal (16). We cannot rule out, however, that "low-dose" methylene blue reached toxic levels and exacerbated red-cell damage in this patient with diminished renal clearance. The recent descriptions of renal failure from phenazopyridine (7, 8, 12–14) necessitate careful scrutiny of the use of methylene blue in this setting.

Physicians should be alerted to the complete spectrum of toxic effects of phenazopyridine. Subsequent treatment with methylene blue in subjects with methemoglobinemia and renal failure should be undertaken only if symptoms other than cyanosis are present or if rapid increases in the blood levels of the oxidized hemoglobin occur. When the administration of methylene blue is necessary, an adjustment in dosage should probably be made for patients with diminished renal clearance.

Acknowledgments

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Granulomatous Hepatitis in Q Fever

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Abstract

Q fever was diagnosed on the liver biopsy specimen from a patient with fever of unknown origin, right-upper-quadrant discomfort, and elevated liver enzyme activities. Granulomas with a fibrinoid ring are characteristic for this disease and must be recognized by the pathologist.

Many patients with granulomatous hepatitis manifest the condition by fever of undetermined origin (1). Granulomatous hepatitis may be associated with numerous disease processes and causes (1–3). Among these, tuberculosis and sarcoidosis are diagnosed most frequently (1, 3, 4), although many infections, systemic illnesses, liver diseases, and drugs have been incriminated. A recent review suggests that drug-induced granulomatous hepatitis occurs in 29% of cases (2). Nevertheless, a large number of cases, ranging from 6% to 36%, are of undetermined etiology (1, 5–8). This may result from the inability to culture or stain the causative agent or make the proper clinicopathologic diagnosis. Granulomas in the liver in Q fever, in the absence of stainable *Coxiella burnetii,* the causative agent, have distinctive features. We recently encountered such a case, in which the diagnosis of Q fever was suggested on the basis of the characteristic granulomatous inflammation and was subsequently confirmed serologically.

Case Report. A 52-year-old white man was admitted to The Mount Sinai Hospital with sore throat, headache, myalgia, fever, and chills of 11 days' duration. He was treated with aspirin and acetaminophen, but remained febrile. His medical history was unremarkable. He had traveled to Egypt two months prior to his illness, but denied contact with farm animals. On admission, he was febrile with a temperature of 102°F and mild right-upper-quadrant tenderness. There was no hepatosplenomegaly. No other physical findings were remarkable. Pertinent laboratory data included a leukocyte count of 7,200/ml with 4% bands, 82% neutrophils, 8% lymphocytes, and 6% monocytes. SGOT was 75 mU/ml (normal 1–50), SGPT 180 mU/ml (normal 1–53), and alkaline phosphatase activity 358 mU/ml (normal 30–90). Antinuclear antibodies, rheumatoid factor, PPD test, antibody to hepatitis A virus, hepatitis B surface antigen, ASLO, monotest, and blood cultures were negative. Sonogram showed a lesion in the left lobe of the liver consistent with a cavernous hemangioma. Chest x ray was unremarkable. Liver biopsy was performed.

Liver Biopsy Findings. The liver showed mild steatosis, with macrovesicular fat globules in scattered hepatocytes throughout the lobules. Many small, well-circumscribed granulomas were noted intralobularly. These granulomas consisted of round or oval aggregates of histiocytes with an outer layer of lymphocytes and eosinophils (Fig. 1). Characteristically, many of the granulomas contained a clear central space, resembling a fat vacuole (Fig. 1), and a fibrinoid ring around the aggregated histiocytes. The eosinophilic fibrinoid ring was rather inconspicuous on hematoxylin-eosin staining (Fig. 2), but was clearly visible and dark red after Masson's trichrome staining (Fig. 3). Direct immunofluorescence studies after deparaffinization of routine

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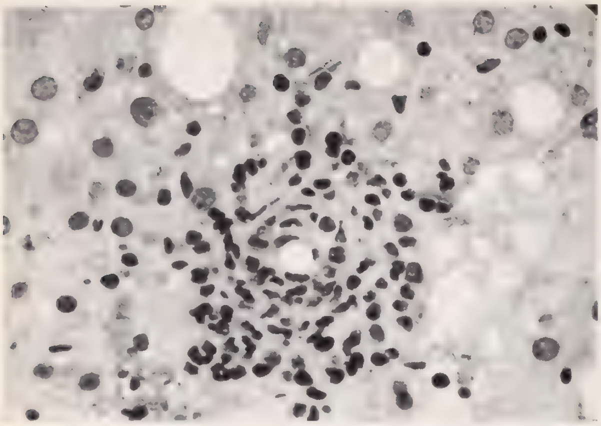
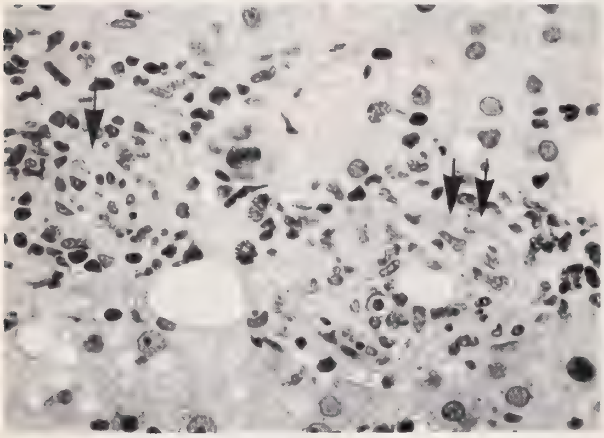
sections demonstrated the presence of fibrinogen in the ring (Fig. 4). Some of the granulomas without a fibrinoid ring contained fibrinogen throughout the lesion (Fig. 4). In a few areas, sinusoids away from the granulomas showed fibrinogen deposits. Although hepatic necrosis was not conspicuous, liver cells showed mitoses, and Kupffer cells were hyperplastic. The presence of the typical granulomas suggested Q fever.

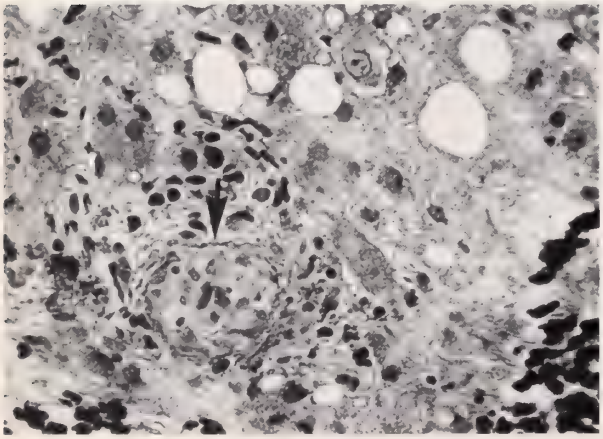
The diagnosis was confirmed by a positive complement fixation test to *Coxiella burnetii* at a dilution of 1:256. Treatment with tetracycline was started, and the patient became afebrile and asymptomatic in one week.

Discussion. Q fever is an acute febrile disease caused by the rickettsia *Coxiella burnetii,* which is usually transmitted by inhalation. In the original description of Q fever, Derrick (9) noted liver involvement in only one of nine cases and emphasized the pulmonary involvement of the disease. Liver abnormalities, such as hepatomegaly, liver tenderness, and jaundice, as well as elevated enzyme activities, were subsequently reported (10–12), but no liver pathology was recorded until 1956. Focal hepatitis with scattered infiltration of polymorphonuclear leukocytes, lymphocytes, and macrophages (13, 14), as well as extensive hepatic necrosis (15), was reported. Granulomatous reaction in the liver was first described in 1960 by Picchi et al (16). Powell documented the liver biopsy findings in 14 patients; the predominant histologic pattern was focal inflammation, ranging from small collections of mixed inflammatory cells to frank granulomas with central necrosis (17).

In 1965, Bernstein et al described, in 2 cases, eosinophilic necrosis in the wall of the sinusoids, sometimes associated with granulomas (18). In 1980, Pelligrin et al studied 17 patients with serologically diagnosed Q fever and found granulomas in all, and so-called "typical" lesions in 14 of them. The "typical" lesions consisted of a granuloma with fibrinoid ring and clear central space







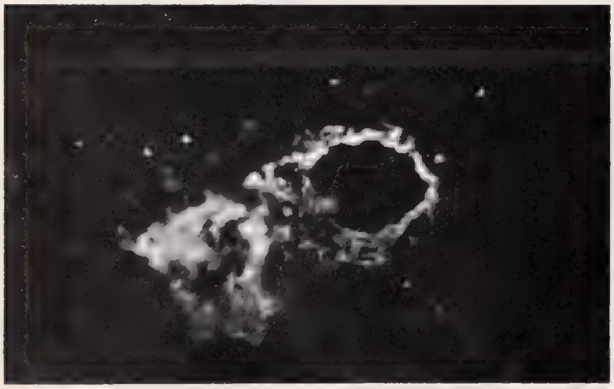


Fig. 1. Liver showing steatosis and small granuloma with central clear space resembling a fat vacuole (H&E, ×250).

Fig. 2. Liver showing two granulomas. Smaller granuloma contains no clear vacuole and has thin, inconspicuous fibrinoid ring (arrow). Larger granuloma displays both central vacuole and fibrinoid ring (double arrows) (H&E, ×250).

Fig. 3. Fibrinoid ring is seen clearly as dark-red ring (arrow) on Masson's trichrome stain (×250).

Fig. 4. Two granulomas showing two different patterns of fibrinogen deposition, diffusely within granuloma or forming ring around it (direct immunofluorescence staining, ×250).

(19). The pathogenesis of this characteristic granuloma formation, also observed in the bone marrow (20, 21) of patients who have Q fever, is not clear. The results of our immunofluorescence studies suggest that fibrinogen is deposited diffusely within a granuloma, and perhaps as the granuloma "matures," the fibrinogen is displaced to the periphery of the lesion and forms a fibrinoid ring (Fig. 4). The large amounts of fibrinogen in these small granulomas in Q fever may be explained as a histiocyte response to the intrasinusoidal deposition of fibrinogen (19, 22). The central clear space was believed to be a fat vacuole incorporated in the developing granuloma (19). Qizilbash in 1983 suggested the diagnosis of Q fever in 5 patients with fever of unknown origin, based on these "typical" granulomas, and was able to confirm the diagnosis serologically in all (22).

Given the worldwide distribution of Q fever, it is important that the pathologist be familiar with the distinctive granulomas in the liver of patients with Q fever in order to arrive at the correct diagnosis.

Acknowledgments

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Severe Upper-Airway Obstruction caused by Rhinoscleroma-Like Granuloma

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Abstract

A Guyanese male who had fever and cough six months before admission developed wheezing during exercise. He had no other symptoms, and normal physical examination at rest. The flow-volume curve revealed the characteristic pattern of fixed upper-airway obstruction, caused by a chronic inflammatory subglottic mass. Although bacteriologic, serologic, and pathologic examinations failed to identify an infectious agent, there was dramatic improvement following treatment with tobramycin, suggesting that the mass was a rhinoscleromalike granuloma. Antibiotic therapy should therefore be considered in managing such lesions even when a causative agent cannot be identified. Delay, or withholding antibiotics, may yield an unfavorable outcome since healing with fibrosis appears to be the natural progression of this type of granuloma.

The symptoms of chronic upper-airway obstruction (CUAO) resemble exercise-induced asthma. Strictures following tracheal intubation, vocal cord paralysis, and tumors account for the majority of cases of such obstruction reported in the literature. Infectious causes have included tuberculosis, syphilis, mycoses (1), and rhinoscleroma (2–4). In this report a patient who had clinical symptoms resembling exercise-induced asthma is presented. The maximum expiratory flow-volume (MEFV) curve revealed the characteristic pattern of fixed upper-airway obstruction, which directed attention to an anatomic abnormality. A chronic inflammatory mass was found, and treatment with tobramycin resulted in complete cessation of symptoms and marked improvement in airway function.

Case Report. An 18-year-old Guyanese male who had been in the United States for six months was admitted to City Hospital Center at Elmhurst because of cough and wheezing. He had been well until six months before admission when he developed fever and cough without expectoration. The patient received ampicillin. The fever abated, but the cough progressed, and over the next two months he developed wheezing.

Wheezing occurred only during exercise, was promptly relieved by rest, and was not precipitated by cold air. He never experienced shortness of breath at rest. He had no known allergies or family history of allergic diseases. He could not discriminate between expiratory and inspiratory difficulty during wheezing. The cough was intermittent and dry and was not associated with pain in the chest or laryngeal area. The patient had lost 20 lb during this illness. He never smoked cigarettes or used illicit drugs. Physical examination revealed an alert male in no respiratory distress. The respiratory system was normal at rest. There were no other abnormal findings.

Laboratory data: Complete blood count, urinalysis, and routine serum chemistry determinations, including liver function tests, were normal. Arterial blood gases breathing room air revealed pH 7.35, PCO_2 47 torr, PO_2 89 torr, and HCO_3 26 mEq/liter. Multiple sputum specimens failed to yield pathogenic bacteria, acid-fast bacilli,

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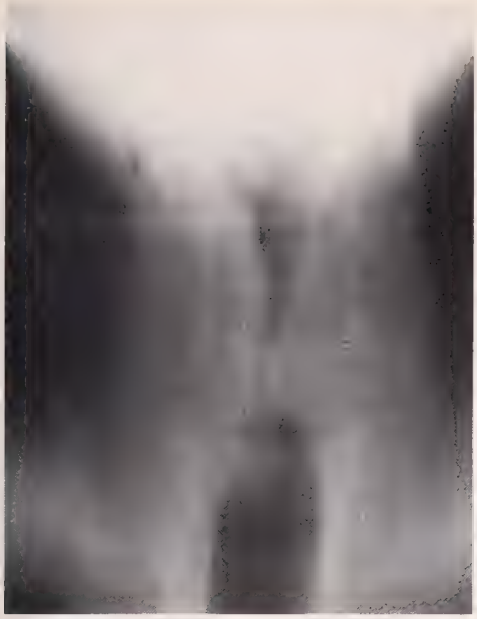

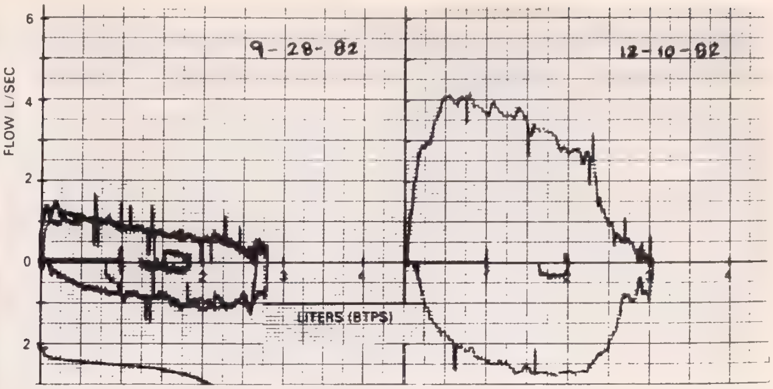

Fig. 1. Left Tomogram of upper airway showing concentric narrowing below level of vocal cords.

Fig. 2. Above Flow-volume curves before (**left**) and after (**right**) treatment, illustrating severe fixed upper-airway obstruction that improved with therapy.

or fungi. There were no malignant cells on cytologic examination. VDRL was nonreactive; complement fixation tests for histoplasmosis and blastomycosis and antinuclear antibodies were negative. The tracheal air column on a chest roentgenogram had significant narrowing. Tomograms of the larynx (Fig. 1) confirmed that approximately 3 cm of the proximal trachea was concentrically narrowed, being about 2 mm in diameter at the narrowest point. Computerized axial tomography of the neck revealed a 2.5- to 3-cm low-density lesion inferior to the hyoid bone. The tracheal lumen was seen left of the midline and was asymmetrical owing to the mass lesion on the right lateral and posterior aspects.

Before treatment (Table, 9/28/82), the patient's vital capacity and maximal expiratory flow rates were markedly decreased. The MEFV curve showed the characteristic pattern of fixed upper-airway obstruction (Fig. 2). The slope of phase III of the single-breath oxygen (SBO_2) test was normal. Surprisingly, the $He\text{-}O_2$ test failed to reveal significant density dependence of expiratory flow at any point in the vital capacity. The $\Delta \dot{V}_{max}50$ (Table) was normal.

After the diagnosis of upper-airway obstruction was established, flexible laryngoscopy was performed. Anterior and posterior rhinoscopy were normal. The right vocal cord was in the midline and was immobile. There was concentric narrowing of the subglottis (lumen approximately 1 mm), and therefore a tracheostomy was performed before the biopsy procedure. Direct laryngoscopy with a rigid laryngoscope confirmed the previous findings; the subglottic mass was firm, cartilaginous, and was covered with pale gray mucosa. There was no evidence of pus.

Two specimens, each measuring 2–4 mm, were obtained from representative areas of the mass; hematoxylin and eosin stains revealed hyperplastic granulation tissue, areas of fibrosis, and chronic inflammation. There were no Mikulicz cells, Russell bodies, or bacteria. Acid-fast stain and PAS stain did not reveal pathogenic organisms. Antibody test for *Klebsiella rhinoscleromatis* was not performed.

Although evidence of bacterial infection could not be confirmed, tobramycin was administered while electron microscopic examination of the specimens was being performed.

In response to this antibiotic, the patient experienced a rapid and significant improvement in breathing during ambulation, and the tracheostomy was removed. He received tobramycin for approximately 30 days and upon discharge was given trimethoprim/sulfamethoxazole for another month. Following discharge he was able to participate in active sports without any symptoms.

Pulmonary function tests (Table, Fig. 2) revealed a significant improvement in vital capacity and all expiratory flow rates. However, the MEFV curve continued to demonstrate evidence of fixed upper-airway obstruction, albeit to a lesser degree. Arterial blood gases showed pH 7.42, PCO_2 37 torr, PO_2 94 torr, and HCO_3 24 mEq/liter. At this time there was evidence of significant density dependence of expiratory flow (Table). Electron microscopy failed to reveal any

TABLE
Pulmonary Function Data before and after Treatment

	9/28/82		12/28/82	
	Observed	% Pred.	Observed	% Pred.
FVC (L)	2.81	56	3.04	60
FEV_1 (L)	1.37	33	2.71	65
FEV_1/FVC (%)	48	—	89	—
PF (L/Sec)	2.11	26	4.18	51
$\dot{V}_{max}50$ (L/Sec)	0.7	11	3.24	53
$\dot{V}_{max}25$ (L/Sec)	0.55	15	2.54	71
FEF 50/FIF 50	0.66	—	1.15	—
ΔN_2 (%/L)	0.7	—	1.0	—
$\Delta\dot{V}_{max}50$ (%)	6	—	34	—

FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; PF, peaked expiratory flow; $\dot{V}_{max}50$ and $\dot{V}_{max}25$, maximal expiratory flow at 50% and 25%, respectively, of vital capacity; FEF 50/FIF 50, ratio of maximal expiratory and inspiratory flow at 50% of vital capacity; ΔN_2, increase in nitrogen concentration of alveolar plateau; $\Delta\dot{V}_{max}50$, increase in flow breathing helium-oxygen mixture as percentage of flow breathing air.

evidence characteristic of rhinoscleroma. The patient continued to be asymptomatic even during strenuous physical exertion.

Discussion. *Clinical features:* The detection of an inflammatory mass that responded dramatically to tobramycin was evidence of a gram-negative infectious etiology. The prolonged history and the presence of areas of fibrosis implied chronicity; therefore rhinoscleroma appeared the most likely diagnosis.

Rhinoscleroma, caused by *K. rhinoscleromatis,* is endemic to Central America and Eastern Europe (5), although sporadic cases have been reported in the continental United States (6). Clinically, an initial stage of rhinorrhea, hoarseness, or dyspnea is followed by granulomatous lesions of the respiratory tract, especially the nose, pharynx, and larynx. The sclerosing nature of the lesion eventually leads to deformities and stenosis of the involved areas. Although Mikulicz cells are characteristically found in large numbers (5, 7), the microscopic appearance may be less characteristic in advanced lesions (5). Gram-negative rods may be demonstrable and *K. rhinoscleromatis* may be cultured from tissue specimens; however, failure to isolate the organism does not exclude the diagnosis (5). Streptomycin, tetracycline, chloramphenicol (4), and topical acriflavine (8) have been used in the treatment with some success in arresting the progression of the disease. Many clinical features and the country of origin of this patient point to rhinoscleroma. However, failure to demonstrate the characteristic Mikulicz cells or organisms in tissue and the absence of involvement of the nasal cavity or the pharynx compel us to diagnose this lesion as a "rhinoscleromalike granuloma" caused by a gram-negative organism.

Pathophysiologic features. Organic lesions (as found in this patient) as well as functional abnormalities of the upper airway may mimic bronchial asthma (9, 10). Miller and Hyatt (11) have stressed that since these entities are uncommon, they may be overlooked in the differential diagnosis of dyspnea and wheezing. These authors pioneered the use of flow-volume curves in the detection of various types of upper-airway obstruction. This report illustrates that the MEFV curve is the best simple test that distinguishes obstruction above the tracheal carina from diffuse airway obstruction. A recent report (12) has shown that a normal MEFV contour may be seen in patients with functional laryngeal narrowing. In contrast, the demonstration of a nearly rectangular flow-volume contour, as shown here, can be considered pathognomonic of fixed upper-airway obstruction.

The work of Gamsu et al (13) has shown that the diameter of tracheal narrowing may be estimated from the FEF 50 obtained via a flow-volume loop. Using their illustration, it could be estimated that the airway lumen was less than 3 mm in diameter in this patient. This is in accord with the naked eye and radiographic estimation of the subglottic opening.

Severe narrowing of the upper airway may cause hypercapnea due to decreased minute ventilation (14, 15). This patient demonstrated hypercapnea associated with a normal A-a gradient before treatment and became normocapnic after improvement in the degree of airway obstruction. Since the SBO_2 test yielded a normal alveolar plateau, attesting to the integrity of airway function during a slow vital capacity maneuver, the resting hypercapnea can be attributed entirely to hypoventilation secondary to severe upper-airway obstruction.

The absence of a flow response to decreasing density of expired air was a surprising finding. Lavelle et al (16) have shown that a high helium response can be a useful method of detecting upper-airway obstruction. However, since the magnitude of turbulence is proportional to the velocity of flow, it is likely that the absence of density dependence was a function of the very low flow rates before therapy. Following an increase in flow rates due to a decrease in obstruction, a significant density dependence of expiratory flow was observed, implying the presence of turbulence across the subglottic narrowing.

Although a low FEV_1, normal SBO_2 test, and hypercapnea with a normal A-a gradient may suggest the diagnosis of upper-airway obstruction, the flow-volume contour is the simplest and most informative test that can confirm the diagnosis during pulmonary function evaluation.

This report demonstrates that potentially curable inflammatory lesions occur in the upper airway and mimic exercise-induced asthma. Although these granulomatous lesions may be caused by pathogenic organisms, bacteriologic identification of the offending pathogen may not always be successful (5). Delay in therapy while awaiting confirmation of the diagnosis, or withholding therapy because no pathogen has been identified may lead to an unfavorable outcome because healing by fibrosis appears to be the natural history of untreated lesions. Therefore, antibiotic therapy effective against gram-negative organisms—which may yield a favorable outcome (as in our patient) even though a causative organism was not identified—should be administered.

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Gastroenterology Notes *David B. Sachar, M.D., editor*

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Two previous articles in this column have assessed the clinical applications of GI motility testing and of new developments in drug therapy. In this third article of our series, Dr. Peter H. Rubin, Deputy Director of the GI Endoscopy Unit, takes a hard critical look at the uses and abuses of upper gastrointestinal endoscopy. In a lively, literate, and refreshingly iconoclastic review, Dr. Rubin provides some sharp insights and some sorely needed common sense in his evaluation of many highly touted new endoscopic techniques.

This essay is must reading for students and practitioners of gastroenterology, for disciples of clinical scholarship, and for devotees of good writing.—DBS

Upper Gastrointestinal Endoscopy: Update 1986

Peter H. Rubin, M.D.

Fiberoptic endoscopy has revolutionized the practice of clinical medicine. It provides a rapid, relatively safe, direct, accurate view of the luminal surface of the gastrointestinal tract. It can be applied to both diagnostics and therapeutics in adults, children, and infants (1–6). In an outpatient setting, without x-ray exposure, the esophagus, stomach, duodenum, and entire colon can be scrutinized, lesions biopsied, and most polyps resected. If an x-ray unit with fluoroscopy is available, the biliary and pancreatic ducts can be filled with dye under endoscopic guidance and studied. With brief hospitalization, esophageal varices can be sclerosed, obstructing tumors stented (7) or decimated by laser (8–10), ulcers and other bleeding sites coagulated (11), papillas of Vater enlarged (12), gallstones removed (12), feeding gastrostomies created (13), foreign bodies extricated (14), and colonic volvuli untwisted (15).

Clearly, the endoscope is a *deus ex machina.* Yet its use is expensive and occasionally dangerous (16–21), and it cannot be applied indiscriminately. Much of the endoscopic literature is descriptive and euphoric, presenting uncontrolled series of successful endoscopic tours de force. As a result, trainees in gastroenterology today are at risk of emerging as aggressive endoscopic "plumbers" rather than as thoughtful clinicians who have appropriately integrated the wonders of endoscopy with the classical tools of history taking, physical examination, and interpretation of clinical laboratory, x-ray, sonography, and radionuclear imaging.

Despite a plethora of literature, only in several areas has there been a critical assessment of endoscopy relative to other, more established, less expensive, and safer diagnostic and therapeutic modalities. This review will look at three areas in which most of the recent literature in upper endoscopy has been concentrated.

GI Bleeding. Much ink has been spilled in establishing the superiority of endoscopy to radiology in the accurate diagnosis of the source of upper gastrointestinal bleeding (22–25). Unfortunately, these studies constitute a victory more for endoscopists over their radiologic colleagues than for patients over their gastrointestinal hemorrhages. Since at least 80% of GI bleeders cease spontaneously, endoscopy-facilitated early diagnosis has not been shown to improve mortality, recurrent bleeding, transfusion requirements, or length of hospitalization (26). Thus, more emphasis in the literature has been placed on identifying "high risk" patients. These include those with bleeding esophageal varices (27, 28) and

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perhaps those with visible vessels in ulcers (29–31), although there is some doubt of the existence and validity of these visible vessels (32). Since diagnostic acumen heightened by endoscopy has not led to better outcome, endoscopists have accelerated their efforts to achieve hemostasis. In light of the present fear of transmitting hepatitis or AIDS by transfusion, endoscopists are likely to be even more aggressive in providing not only diagnoses but therapy. These efforts will increasingly utilize electrocoagulation (11, 33–35), lasers (36, 37), thermal probes (38), and direct injections of hemostatic solutions (39). Once this endoscopic therapeutic armamentarium is better established and standardized it will have to be compared prospectively against traditional medical and surgical management to see whether patient outcome is improved.

Esophageal Varices. In recent years enthusiasm has revived for the endoscopic obliteration of bleeding esophageal varices (7, 27, 28, 40–43). This is accomplished by injecting sclerosing agents either intravariceally or perivariceally under direct vision. The modern flexible endoscope is well suited for this purpose, because it can be introduced safely without general anesthesia in patients with highly compromised hepatic function. The endoscope can be maneuvered to bring the tip directly into contact with varices anywhere in the circumference of the esophageal lumen. Bleeding can be seen and sclerosing agents injected through retractable needles in polyethylene sheaths passes through the endoscope.

Controlled prospective trials have established that endoscopic sclerosis of varices (ESV) is superior to conservative medical management in terms of reduced transfusion requirement and incidence of rebleeding (27, 28, 40, 41). It is not universally agreed, however, that ESV improves longevity (28, 40, 42). When compared to surgical portasystemic shunting, ESV results in a lower transfusion requirement and less medical expense, but not in less rebleeding, rehospitalization, or greater longevity (43).

On balance, ESV appears to represent an advance (even though perhaps palliative) over the traditional management of this high-risk group of patients. The procedure does carry its own significant morbidity, including chest pain, esophageal ulceration, stricture, mediastinitis, and pleural effusion (44). Therefore, it cannot be recommended prophylactically in patients with nonbleeding varices, although this use has been suggested (45).

Biliary Endoscopy. Endoscopy of the biliary tree has progressed as it has in other portions of the gastrointestinal tract, from diagnostics to therapeutics. Here, unlike the case of gastrointestinal bleeding, there has been a wedding of the previous warring disciplines of endoscopy and radiology. Thin, flexible, side-viewing instruments have facilitated the cannulation of the papilla of Vater and the injection of radioopaque contrast, producing endoscopic retrograde cholangiopancreatography (ERCP) (12). Further advances in endoscopes have led to intraoperative choledochoscopy (46), potentially lowering the 10%–20% incidence of retained common duct stones (47, 48). Endoscopic accessories have more recently permitted sphincterotomy and the extraction of retained common duct stones (12, 49–52). Considerable expertise is required to perform these interventional endoscopic procedures, and all series report up to 10% of patients suffering complications, including pancreatitis, perforation, cholangitis, or late stricture (50–52).

In patients with malignant biliary stricture, endoscopically placed stents can lead to palliative drainage (12, 53–55). The stents have to be removed or replaced in as many as 20% of the patients due to clogging or sepsis (55), although larger endoprostheses have recently been developed which may improve these statistics (56). In many medical centers the endoscopic insertion of these stents has become the procedure of choice for treatment of patients with biliary tract obstruction who are poor operative risks or have unresectable malignant tumors (55).

Again, however, what is lacking in these enthusiastic studies in the management of gallstones and biliary tumors is controlled comparison with other therapeutic options such as surgery to examine complications, need for further intervention, length of hospitalization, prognosis, quality of life, and expense.

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Radiology Notes

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Skeletal Metastases of Seminoma of the Testicle

GEORGE HERMANN, M.D., ANDREW NOVICK, M.D., AND LINDA CARR, M.D.

Case Report. A 49-year-old white man was admitted to Mount Sinai Hospital for evaluation of progressively increasing lower back pain and an enlarging scrotal mass of eight months' duration. Several days before hospitalization the patient noted numbness and pain in his right foot. An ultrasound examination of the scrotum eight months prior to admission revealed a right hydrocele. His medical history was otherwise unremarkable.

Physical examination revealed a fluid-containing mass, 10 × 15 cm, in the right scrotum. Neurologic examination gave evidence of flexion weakness of his right foot and decreased anal sphincter tone, suggestive of a cauda equina syndrome. Abnormal laboratory values included serum alkaline phosphatase of 141 (normal, 30–110 mU/ml; hemoglobin of 11.3 mg% (normal, 16 ± 2 mg%), and serum hematocrit of 33.6% (normal, 47 ± 5%).

Routine chest roentgenogram was normal. The conventional radiographic examination of the lumbosacral spine revealed a lytic lesion of the sacrum, confirmed by computed tomography (Fig. 1), and an open biopsy of the right sacral lesion was performed. The pathological specimen consisted of fibrolipomatose tissue infiltrated with poorly differentiated malignant tumor cells containing glycogen and clear cytoplasm. Needle aspiration of the scrotal mass drained 500 cc of blood-tinged fluid containing inflammatory cells without evidence of tumor. Subsequent physical examination revealed a large, stony-hard mass contiguous with the right testicle. Ultrasound examination documented bilateral hydroceles with a solid heterogeneous mass arising from the right testicle measuring 7.0 × 5.5 × 7.5 cm (Fig. 2). Bipedal lymphangiograph showed a large defect in a right external iliac lymph node.

The patient underwent right radical inguinal orchiectomy. Pathologic study demonstrated almost complete replacement of the right testis by seminoma extending through the tunica albuginea and invading the lymphatics and the spermatic cord. Microscopic comparison with the sacral biopsy revealed identical tumor cells.

Serum alphafeto protein and human chorionic gonadotropin levels were normal.

The patient underwent a course of 3600 rads of radiotherapy to the sacral area, resulting in the improvement of weakness in his right foot. He was also treated with cis-platinum, Bleomycin, and Velban.

Diagnosis: Seminoma of right testis metastatic to sacrum.

Discussion. Cancer of the testis represents about 1% of all cancers in males, with an incidence of 2 to 3 per 100,000 males per year in the United States (1, 2). The average age of those affected is 32 years. Although the incidence of testicular cancer is low, it is the most frequent cancer in younger men. It has a slight predilection for the right side, and for the cryptorchid testis.

In most patients the initial symptom is a painless mass or swelling of the testicle (3). The primary tumor is usually firm to hard on palpation and may extend along the spermatic cord (3). More than 95% of all solid testicular tumors are malignant (4).

Testicular tumors are either of germinal cell (97%) or nongerminal cell (3%) origin. Germinal cell tumors consist of seminoma, embryonal carcinoma, teratoma, teratocarcinoma, choriocarcinoma, and mixed types. Nongerminal cell

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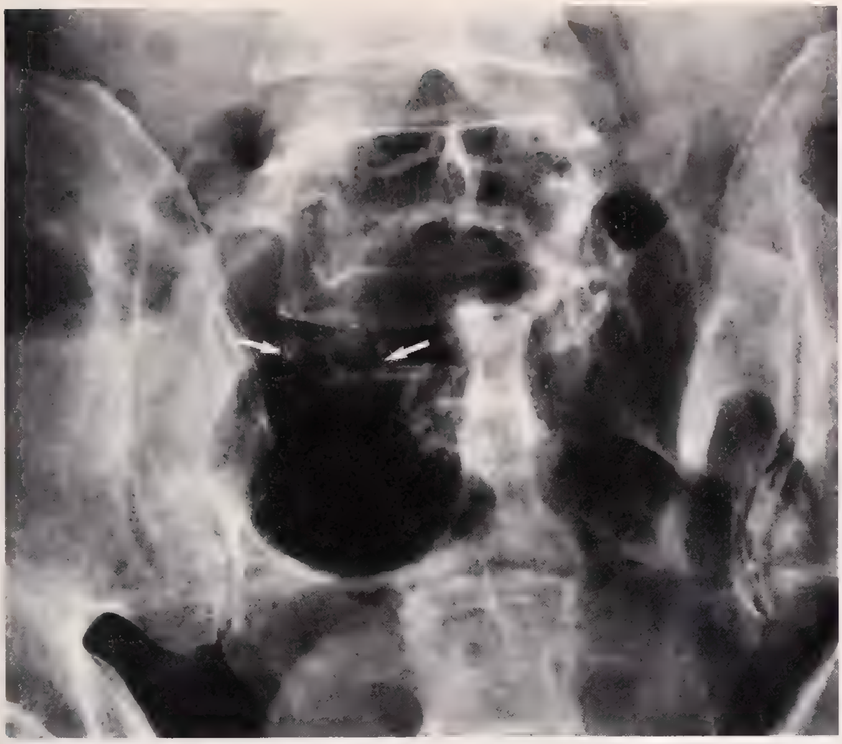

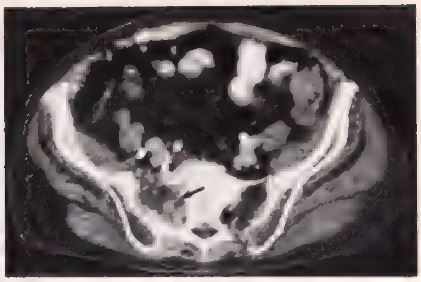

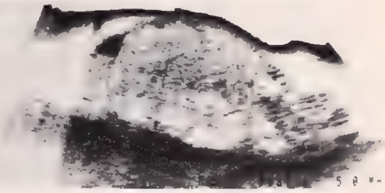

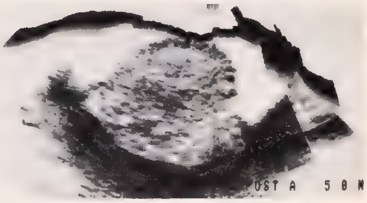

Fig. 1. Plain radiograph (**above**) and CT scan (**upper right**) of sacrum; destructive process involves upper part of right side of sacrum (arrows).
Fig. 2. Transverse (**middle right**) and longitudinal (**bottom**) scan of right testicle, which is enlarged. Normal echo pattern is completely disrupted and replaced by heterogenous solid material. Note large hydrocele.

tumors include interstitial cell and gonadal-stromal tumors.

Seminoma is the most common testicular tumor, comprising approximately 40% of reported cases (1–4). It is usually found as pure seminoma but in 10%–15% of cases is of mixed germ cells, usually embryonal carcinoma (1). Measurements of alphafeto protein and human chorionic gonadotropin are often elevated in embryonal, terato carcinomas, and choriocarcinomas and hence are used in the determination of testicular germ cell tumors. Elevated levels in an apparent pure seminoma should suggest the presence of a mixed tumor, which may change the therapy needed.

Histologically, seminoma is made up of sheets of moderately large and round or polypoid cells with clear, well-defined cytoplasm. Typically it has a fibrous stroma infiltrated by lymphocytes (2, 5). It is a tumor of variable aggressive potential and usually metastasizes via the lymphatics, in contrast to the hematogeneous spread of nonseminomatous testicular carcinomas. The tumor usually spreads linearly from the testicular lymphatics to involve the lumbar paraaortic lymph nodes, and then through the thoracic duct to the left supraclavicular node and subclavian vein (6). Ultimate survival depends mainly on the histology of the tumor and the degree of spread and not on the size of the primary lesion (3). The histologic pattern of metastatic seminoma is identical with the primary lesion in 65% of the reported cases. The remaining patients have shown metastases of mixed germ cell type (3, 6).

Skeletal metastasis from seminoma appears to be unusual; in only a few reported cases has the radiologic diagnosis been made while the patient was still alive.

Johnson et al, reviewing the autopsy material of 19 patients with seminoma, found metastatic involvement of the liver and retroperitoneal lymph nodes (80%) and lung and mediastinum (70%). Skeletal metastases were found in 8 of these patients, although only two patients were known to have such involvement clinically. The high rate of skeletal involvement in this series is probably not truly representative of the overall incidence of skeletal metastasis, because the autopsied patients already had extensive disease. Radiologic diagnosis of skeletal metastasis is uncommon; when it is made, a lytic pattern (6, 7) is seen. The varied cases in which the lesions were detected consisted of involvement of the maxillary antrum, the jaw (5), and the skull (8). In one patient the primary tumor was never found and the only diagnostic clue was a metastatic deposit in the temporal bone.

Summary. Bone metastases from seminoma are rare but may occur subclinically (6). This diagnosis should be considered in younger men when bone pain and a testicular mass are present.

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Hypoplasia of the Lung: CT Appearance

David S. Mendelson, M.D., Phillip Hahn, M.D., Burton A. Cohen, M.D., and Maria L. Padilla, M.D.

Case Report. A 33-year-old woman was admitted to the hospital because of progressive cervical dilatation during a 35-week intrauterine pregnancy. The chest roentgenogram taken at this time (Fig. 1) showed dextroversion of the cardiac silhouette and a normal abdominal situs. On the lateral film the posterior right hemithorax was completely opacified.

Physical examination revealed decreased breath sounds at the right lung base with auscultation of the heart sounds at the right precardium. EKG revealed counterclockwise rotation without any significant abnormality.

The patient's hospital course was unremarkable and she had a normal vaginal delivery at term. She was then referred to the hospital pulmonary clinic for evaluation of her chest findings. A lung perfusion scan demonstrated hypoperfusion of the left upper lung and basal segments of the right lower lung. A CT scan was performed to delineate the thoracic anatomy and to exclude a right-sided chest mass (Fig. 2). It revealed a dextroverted heart, elevation of the abdominal contents, and a diminutive right hilum.

Diagnosis: unilateral pulmonary hypoplasia diagnosed by computerized tomography.

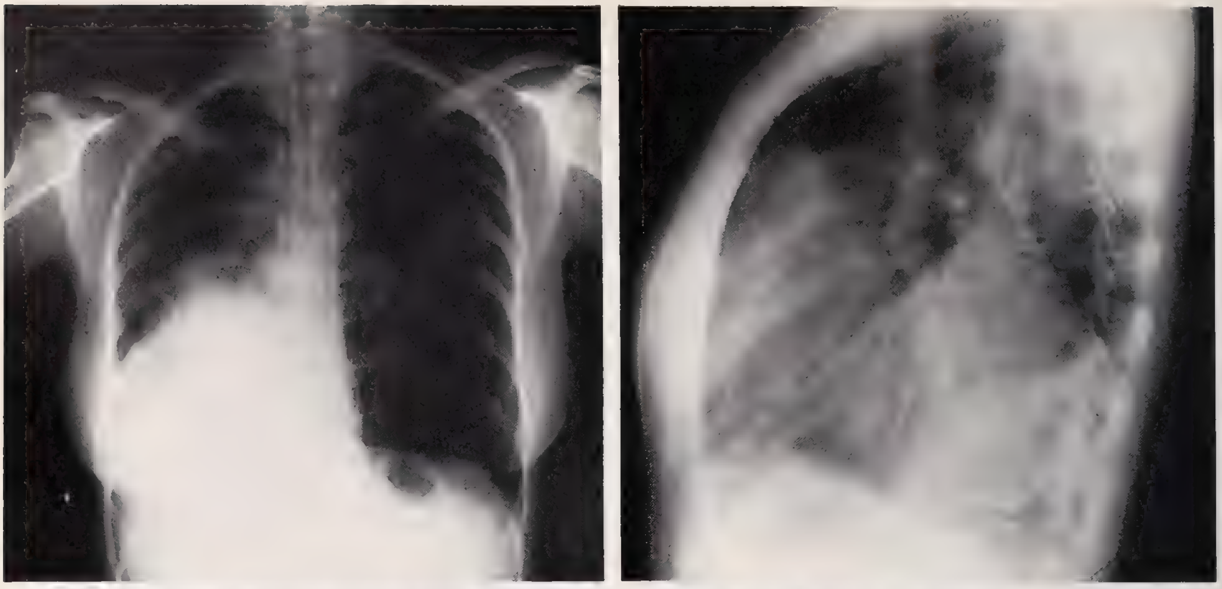
Discussion. Congenital unilateral pulmonary hypoplasia in adults is a well-described phenomena (1–7). Unlike the infant, the adult is often asymptomatic (1-3, 9) and the abnormality is almost always incidentally discovered on chest roentgenogram performed for other indications.

The characteristic radiographic findings of pulmonary hypoplasia include opacity on one side of the chest representing the rotated mediastinum. In addition, the diaphram is often elevated on the ipsilateral side. Despite the volume loss, the bony thorax often remains symmetrical. This feature helps differentiate this congenital anomaly from acquired atelectasis, in which the intercostal spaces are narrowed. Although the main bronchus may be present, only a few orders of branch bronchi, minus distal branches or alveoli, may be present. Any aerated lung on the involved side often proves to be herniated lung from the contralateral side. The anatomy of the existing bronchi may vary from narrowed to ectatic (1–4).

The five lobar bronchi develop in the embryo between days 30 and 32. The degree and anatomy of hypoplasia depends on the time of arrest in bronchial budding. The associated vascular anomalies are believed to be secondary to the abnormality in bronchial branching. The pulmonary vasculature develops about the already existing bronchial buds (5) and in most cases the bronchi as well as the vessels are abnormal. On occasion only the primary vessels are abnormal suggesting that the abnormal development occurred later in embryologic development (6–8). The hypoplastic lung is seen pathologically as a fibrous sheet of tissue about existing bronchi. Nonaerated alveoli may be present as clefts within the fibrous sheet (1).

A variety of other congenital anomalities can be associated with hypoplasia of the lung. These include cardiovascular and bony abnormalities and bronchogenic cysts (7, 9). The well-known "scimitar" syndrome consists of hypoplasia of the right lung associated with an anomalous pulmonary vein draining below the diaphragm. (7, 9).

The case described here demonstrates the value of CT in helping confirm the diagnosis. Previously, invasive techniques—bronchography and angiography—were often used to confirm the diagnosis. In the case presented here, CT clearly identified the dextroverted heart and the elevated abdominal organs and excluded the possibility of a mass in the lung or at the right hilum. Only an endobronchial lesion could not be excluded. An important additional finding in this case was the presence of a diminutive right pulmonary artery, suggesting hypoplasia.



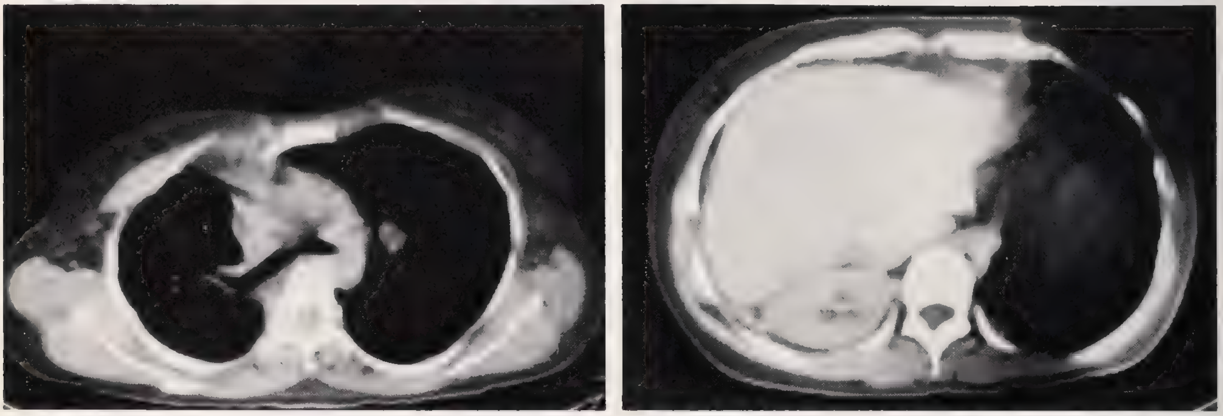


Fig. 1. Posterioanterior (**top left**) and lateral (**top right**) roentgenograms demonstrate dextroversion of heart, increased density posteriorly in right hemithorax, and slight narrowing of right intercostal spaces.

Fig. 2. CT image (**bottom left**) demonstrates diminutive vessels at right hilum as well as volume loss in right hemithorax. Second image (**bottom right**) caudad to the first identifies liver and kidney elevated into right hemithorax, thus accounting for increased density behind heart.

Summary. A classical chest roentgenogram in an asymptomatic patient may strongly suggest the diagnosis of pulmonary hypoplasia. In the past, bronchography and angiography were used to confirm the diagnosis. We believe that CT provides an equally definitive and—more importantly—a noninvasive means of confirmation.

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Gastroenterocolic Fistula Complicating Benign Peptic Ulcer

BRIAN A. KRIVISKY, M.D., STEVEN J. BIER, M.D., AND DAVID G. OSTROLENK, M.D.

Case Report. A 73-year-old white, legally blind woman was admitted to hospital for lower abdominal pain and diarrhea of two weeks' duration. Nine months prior to this admission she was hospitalized for abdominal pain; CT at that time demonstrated an abdominal aortic aneurysm, and a barium enema showed multiple diverticula. The patient was treated symptomatically, did well, and was discharged. The patient had undergone an appendectomy about 50 years previously, was taking antihypertensive medication, and was known to have gallstones. On admission the patient had borderline hypertension, active bowel sounds, and a tender lower abdomen with a suggestion of minimal rebound tenderness. The rectal examination showed brown heme-positive stool but no tenderness. White blood cell count was 11,400, hemoglobin was 11.5 mg%, and hematocrit was 32.4%. The clinical diagnosis was "diverticulitis."

A barium enema was performed 11 days after admission. The abdominal film of that study (Fig. 1) revealed multiple collections of gas beneath the left hemidiaphragm. One represented the splenic flexure, the other stomach, and below the latter a narrow vertical air collection representing the gastric ulcer with perforation into the colon. The subsequent contrast study demonstrated abnormal flow of barium from the transverse colon to the stomach and duodenum associated with an edematous mound in the region of the fistula (Figs. 2, 3).

The patient underwent a laparatomy on day 15. A subtotal gastrectomy and Billroth II anastomosis, fistula closures, resection of fourth portion of duodenum and transverse colon, and cholecystectomy were performed. Pathological examination of the specimen revealed benign peptic ulceration of the body of the stomach with fistulous communication to the colon. The patient did well postoperatively.

Discussion. Gastrocolic fistula in general is rare. Although many causes for it have been described (Table), the most frequent association is with gastric or colonic carcinoma; fewer than 250 cases due to malignant disease (1) have been described. Benign ulcer is an even more unusual cause; only 50 cases (2, 3) have been described. A three-way fistula associated with nonoperated-on benign disease, as in the case described here, is even rarer.

Gastrocolic fistula is associated with a classic triad of symptoms, diarrhea, weight loss, and feculent vomiting. In one case reported due to benign gastric ulcer, the patient endured ten days of feculent vomiting (4). McDaniel et al (5) described a similar complication in Crohn's disease: cold diarrhea after drinking iced tea. Despite the classic triad of symptoms, gastrocolic fistula may occasionally occur with no specific diagnostic symptoms (3). Of all the diagnostic examinations available, the barium colon examination is still by far the most accurate method of diagnosis (1,

TABLE
Etiology of Internal Gastric Fistulas

Malignant
Carcinoma of
  stomach or colon
  (most common cause)
Carcinoid
Hypernephroma
  of left kidney
Lymphoma
Postoperative
Gastrostomy or
  gastroenterostomy
  (common cause)
Medication
Salicylic acid
Steroids

Inflammatory
Benign gastric ulcer
Diverticulitis
Pancreatic disease
Crohn's disease
Radiation enteritis
Intraperitoneal abscess
Ulcerative colitis
Appendicitis
Tuberculosis
Syphilis

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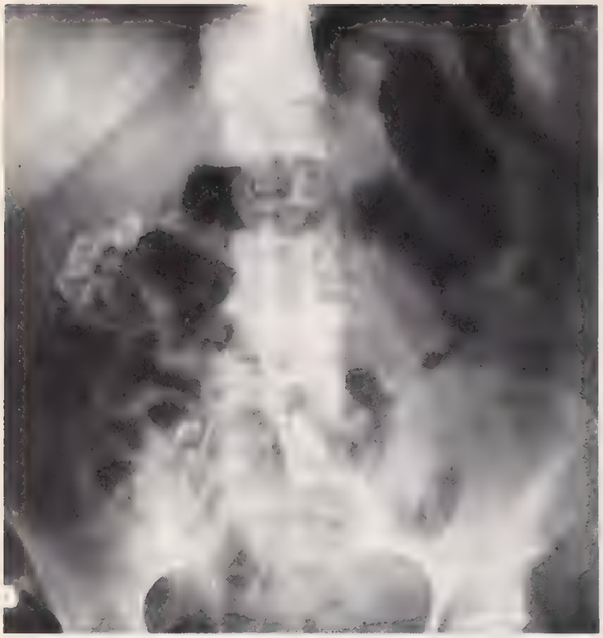

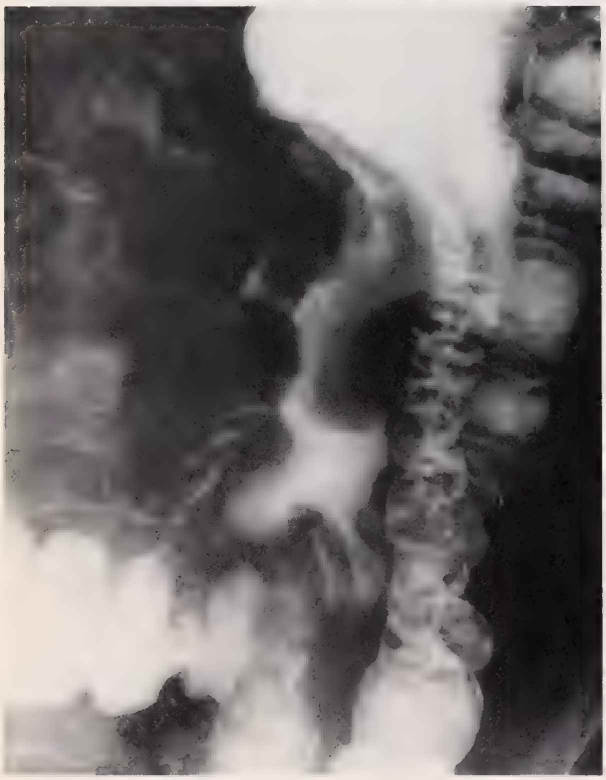

4); it is almost 100% accurate, far more so than either gastroscopy or an upper gastrointestinal series, although this latter is occasionally adequate (6).

Even less common are periduodenal fistulas in benign disease. Three cases of this type, one from stomach to descending duodenum, are described and discussed by Goldstein (7). Maingot's (1) review of gastric fistulas notes that gastrojejunocolic fistulas are complications of marginal ulcers secondary to bypass surgery.

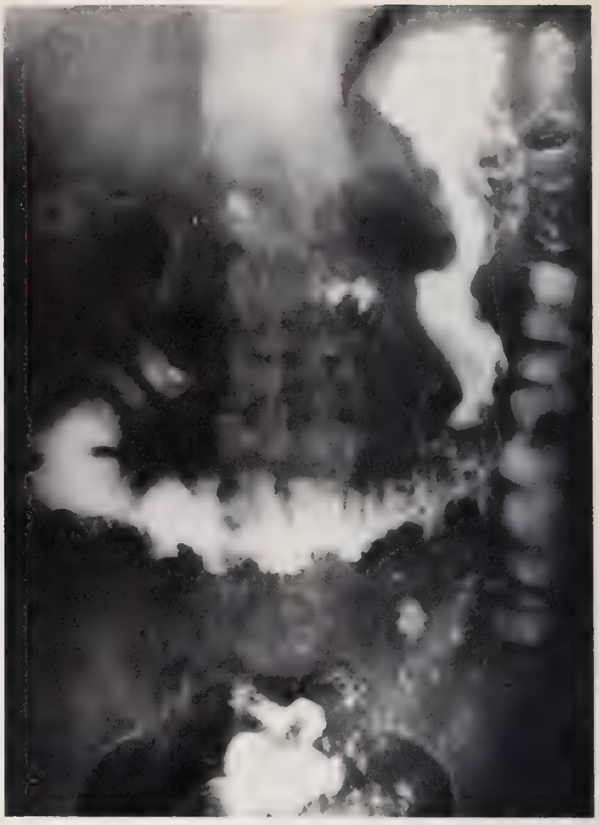

Fig. 1. Above left Multiple gas collections are seen within left upper quadrant. These represent stomach, splenic flexure, and an associated large ulcer located just below stomach. Calcified gallstones are incidentally noted in right upper quadrant.

Fig. 2. Above right Barium colon examination demonstrates fistulous communication between transverse colon, stomach, and duodenum.

Fig. 3. Left More detailed view of fistula demonstrates large ulcer associated with surrounding edema within stomach and colon.

Treatment of gastrocolic or gastroenterocolic fistula is by en bloc resection of the fistulous tract and adjacent involved bowel, partial gastrectomy, and reestablishment of bowel continuity (1, 3).

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Surgical Pathology Notes *Ira S. Schwartz, M.D., editor*

Primary Angiosarcoma of the Heart

JEFFREY A. FREED, M.D., MAMORU KANEKO, M.D., AND RONALD E. GORDON, PH.D.

Abstract

We report a case illustrating the use of peroxidase–antiperoxidase staining for Factor VIII-related antigen in the differential diagnosis of a predominantly solid spindle cell primary sarcoma of the heart. Initially, the pattern of fascicles of spindle cells intersecting in a myxoid vascular stroma led to the diagnosis of vascular leiomyosarcoma. Immunohistochemical staining for Factor VIII-related antigen revealed the endothelial nature of the tumor cells, establishing the diagnosis of angiosarcoma. Electron microscopy demonstrated features typical for angiosarcoma, while ruling out leiomyosarcoma.

Primary sarcomas of the heart are rare. They include angiosarcoma, rhabdomyosarcoma, fibrosarcoma, malignant fibrous histiocytoma, osteosarcoma, leiomyosarcoma, liposarcoma, and synovial sarcoma (1). We report a case of a primary angiosarcoma of the heart, with a predominantly solid histology, the endothelial nature of which was confirmed by demonstrating Factor VIII-related antigen (FVIII-RAg) in the tumor cells with peroxidase–antiperoxidase (PAP) stain (2).

Case Report

A 23-year-old white man was admitted to Huntington Hospital, New York, following a grand mal seizure. Computed tomography (CT) of the head revealed a right temporal and left parietal lesion, each focal and enhanced with contrast, interpreted as hemorrhage into tumor metastases. The patient's seizures continued, and he was transferred to The Mount Sinai Hospital. Physical examination at that time revealed slurred speech, right-upper-extremity weakness, bilateral Babinski signs, and an early diastolic "plop" sound in addition to a II/VI systolic and a II/IV diastolic murmur. The chest x-ray showed a normal cardiac silhouette and clear lung fields bilaterally. A two-dimensional echocardiogram revealed a right atrial pedunculated mass prolapsing through the tricuspid valve and anchored at the interatrial septum. In addition, cerebral angiography showed neovascularization in the lesions discovered on CT, confirming the diagnosis of metastatic tumor. A bone scan revealed a right femoral metastatic lesion. The central nervous system symptoms, compatible with impending brain stem herniation, improved markedly with radiation to the head, as did the lesions on CT. Resection of the right atrial tumor was performed on cardiopulmonary bypass. Intraoperatively, unsuspected lung metastases were found. In addition, tumor infiltrated the atrial septum and the pericardium, with superficial implants on the adventitia of the pulmonary artery trunk.

Chemotherapy was initiated, and the patient was discharged two weeks postoperatively. He had recurrent symptoms of increased intracranial pressure and died one week after discharge. Consent for an autopsy was not granted.

Pathologic Findings

The main surgical specimen consisted of a large, highly bosselated, pedunculated mass, 9.0

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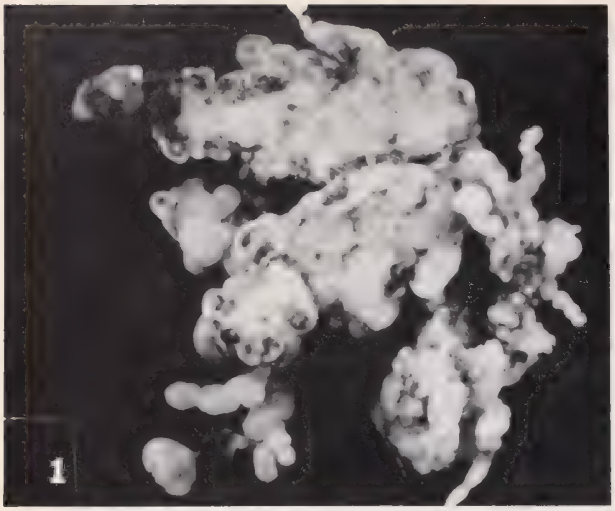


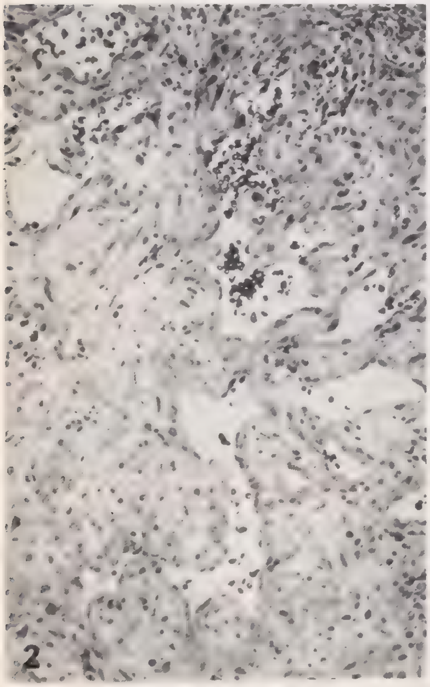
Fig. 1. Gross photograph of main tumor mass. External aspect is bosselated and covered by numerous small serous cysts. Cut surface is solid, yellow-tan, and firm, with focal hemorrhage.

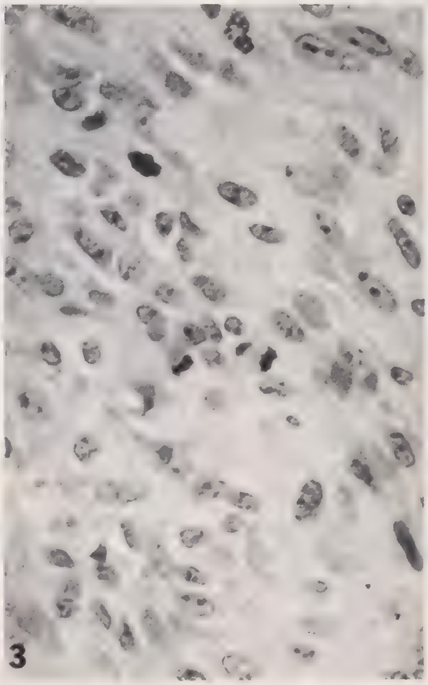
× 2.0 × 1.0 cm, with an external aspect completely covered by innumerable cysts containing mucoid material and measuring up to 0.8 cm in diameter, in addition to firm, solid, white nodules of the same dimensions (Fig. 1). The cut surface was mostly solid, yellow-tan, and firm, with focal hemorrhage.

Microscopically, numerous irregularly anastomosing vascular channels lined by plump, hyperchromatic endothelial cells were present (Fig. 2). However, throughout most of the specimen, a solid spindle cell pattern predominated (Fig. 3). Fascicles of spindle cells, with oval nuclei and eosinophilic cytoplasm, intersected at right angles. Some areas showed a mitotic rate as high as 5 per 10 high-power fields. The nuclei in such areas were plump and hyperchromatic and contained one or two nucleoli with a dispersed chromatin pattern. Extensive areas of necrosis and hemorrhage were seen.

A reticulum stain neatly outlined the basement membrane along the antiluminal aspect of the endothelial cells in normal vessels as well as vascular slits in the tumor. A vascular pattern was also demonstrable in the solid areas of tumor. A trichrome stain failed to show myofibrils in the cytoplasm of the spindle cells. The FVIII-RAg stain (PAP technique) produced a granular brown staining in the cytoplasm of the endothelial cells lining normal vessels and tumor vascular slits and stained a large fraction of the spindle cells occupying solid areas of the tumor (Fig. 4). The solid areas stained quite strongly.

Electron microscopy was performed on representative areas of the tumor, which had been glu-





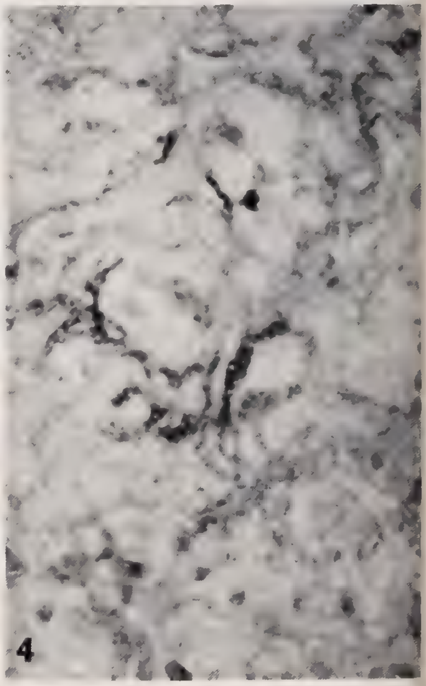


Fig. 2. **Left** Photomicrograph showing irregularly anastomosing vascular channels lined by plump, hyperchromatic endothelial cells (× 100 original magnification). **Fig. 3. Center** Photomicrograph showing solid spindle cell pattern, which constituted majority of tumor. Cells show oval nuclei with eosinophilic cytoplasm and numerous mitotic figures. One or two nucleoli and dispersed chromatin pattern are seen (× 400 original magnification). **Fig. 4. Right** Peroxidase-antiperoxidase stain for Factor VIII-related antigen showing granular brown positively staining tumor cells even in solid areas of tumor (× 400 original magnification).

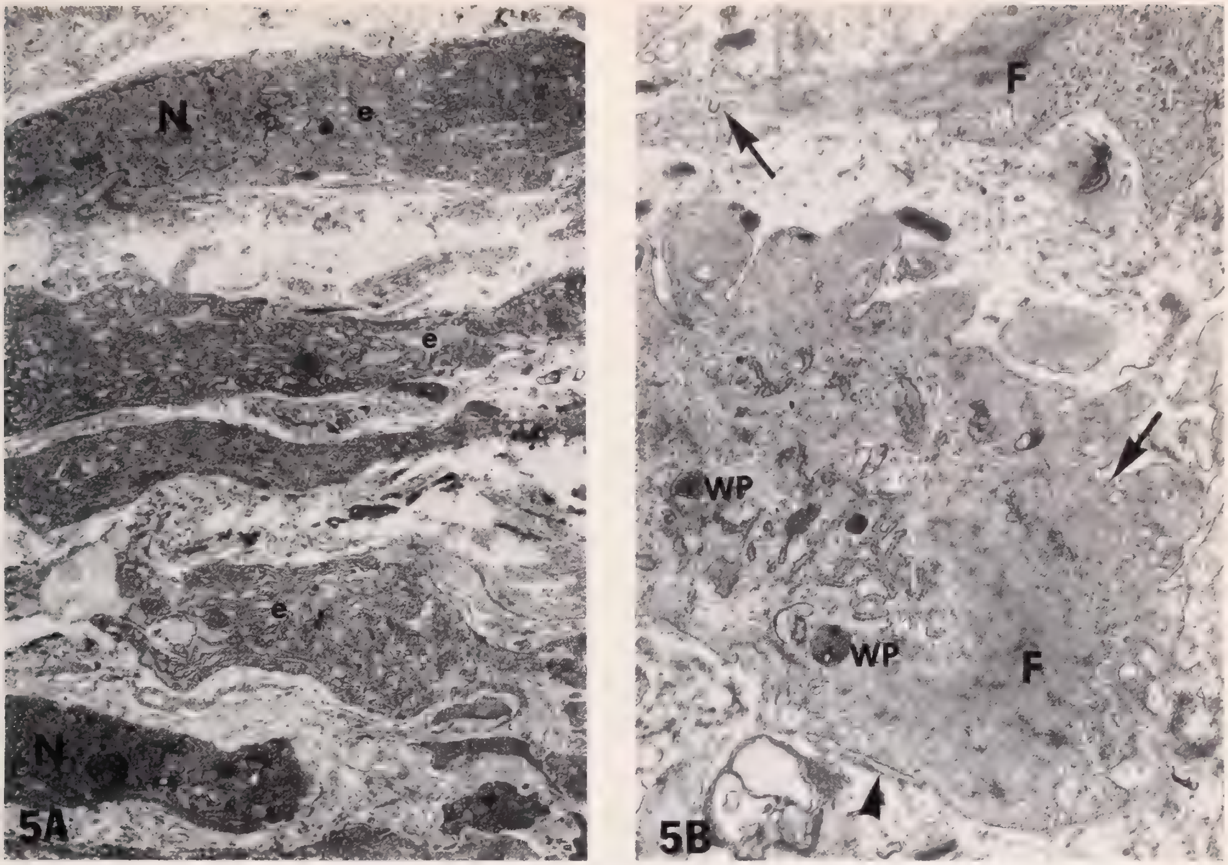


Fig. 5. **A,Left** Electron micrograph of cardiac mass at low magnification reveals homogeneous population of long spindle cells in parallel array. Cells show large oval indented nuclei (N) with significant amount of heterochromatin throughout nucleoplasm and single prominent nucleolus. Cells contain large amount of granular endoplasmic reticulum (E), much of it dilated. (×4,300). **B,Right** At higher magnification, cells contain many microfilaments, some aggregated into structures resembling dense bodies (F). Many Weibel-Palade bodies (WP) are seen throughout cytoplasm, with many polyribosomes. Patches of basement membrane (arrowhead) and pinocytotic vesicles (arrows) were randomly distributed along cell perimeter. (×15,000)

taraldehyde-fixed, dehydrated in ethanol, and embedded in Epon 812. Micrographs revealed a single population of spindle cells, many of which lined vascular spaces (Fig. 5A). A discontinuous basement membrane was observed on one surface of the cells, frequently adjacent to hemidesmosomes on the cell (Fig. 5B). Pinocytotic vesicles were a common feature around the entire cell periphery (Fig. 5B). The cytoplasm of these cells contained a significant amount of granular endoplasmic reticulum, much of which was dilated; polyribosomes; intermediate 10 nm microfilaments; a few lipid vacuoles; and numerous electron-dense lysosomes (Weibel-Palade bodies) (Fig. 5B). These ultrastructural features further support an endothelial cell origin.

Discussion

Angiosarcoma is probably the most common primary cardiac sarcoma (1). More than 60 cases have been reported in the world literature, the patients ranging in age from 9 to 76 years (1, 3). There is a two- to three-fold male predominance, most cases occurring in the 20-to-50-year age group (1). Since the right atrium is distinctly the most common site of origin, the clinical presentation is quite uniform, with congestive heart failure and obstruction to venous return resulting in congestion of viscera and edema of the face (4). Many tumors involve the pericardium, with consequent pericardial effusion. Rarely, recurrent bloody pericardial effusion may mimic tuberculous pericarditis. Systolic murmurs and atrial arrhythmias are associated clinical findings.

Laboratory abnormalities are usually confined to elevated transaminase levels, owing to chronic passive congestion of the liver in cases complicated by pericardial tamponade (3, 5). The chest roentgenograms almost invariably show a globular massive cardiac silhouette and occasionally show right ventricular outflow tract prominence or pulmonary or rib metastases (6, 7). Electrocardiographic abnormalities include nonspecific ST-T wave changes, low-voltage complexes, right-axis deviation, and atrial arrhythmias (7). The prognosis is poor, most patients succumbing within one year (1). With cobalt-60 irradiation, cyclophosphamide, and vincristine, a few patients have survived without metastases or progression of cardiac tumor, with a follow-up in one patient of three years (4, 6). In our case, seizure was the initial symptom. We found no other cases of patients with central nervous system symptoms other than syncope (3). Brain metastases are reported in only four cases (7). Another unusual finding in our case was the normal chest film on admission, also not reported in the literature.

The gross pathologic description of primary cardiac angiosarcoma usually includes right atrial intracavitary projections up to 12 cm in diameter, obstructing one or both venae cavae or the tricuspid valve (6, 7). The gross appearance has been infrequently described in the literature, but lobulation and focal hemorrhage have been seen, with variegated color from yellow to glassy-gray (6). Microscopically, malignancy is determined by the presence of irregularly anastomosing vascular channels; foci of endothelial cell aggregates, lacking intervening stroma, forming tufts that project into vascular spaces; and spindle cell areas (1). Pleomorphism, anaplasia, and mitoses are of secondary importance in determining malignancy (1). Cardiac angiosarcomas typically exhibit irregularly anastomosing vascular channels in a dense cellular background (4). The channels are lined by plump or elongated pleomorphic cells and sometimes contain thrombi (4). Typically, plump endothelial cells bulge into vascular lumens, giving the appearance of a solid pattern (1). In solid areas, cords of spindle cells are often separated by myxoid extracellular material (3). Different portions of a tumor may vary considerably in composition, some appearing mostly vascular while others appear mostly solid (7). Although electron microscopic studies of primary cardiac angiosarcomas are lacking, angiosarcomas from other sites show spindle cells with irregular nuclear borders and one or two nucleoli, many ribosomes, pinocytotic vesicles, fine processes, primitive desmosomes or hemidesmosomes, a well-defined basal lamina, intermediate filaments (vimentin type), and, rarely, Weibel-Palade bodies (8–10). Immunohistochemistry has not been used to study primary cardiac angiosarcoma in the published reports. Nonetheless, several authors discuss the use of a PAP stain for FVIII-RAg in angiosarcomas from other sites (11–14). Despite conflicting reports, most angiosarcomas show positive staining for FVIII-RAg, with solid areas staining in a majority of cases, though usually less strongly than endothelial cells lining vascular channels (11, 13, 14). More recently, *Ulex europaeus* I lectin has emerged as a superior endothelial cell marker, yielding a positive result in 27 of 27 angiosarcomas studied by Ordoñez and Batsakis (11).

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Students' Corner

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Acute Acalculous Cholecystitis: A Limited Review of the Literature

KANT YUAN-KAI LIN, M.D.

Gallstones are present in 95% or more of acute cholecystitis cases. Acute acalculous cholecystitis (AAC) has been described in the setting of trauma, postsurgery, burns, anesthesia, massive transfusions, sepsis, shock states, long-term parenteral nutrition, and in children (1–15). In these situations it appears that the incidence of AAC is much greater than the 5% generally cited for the general population. Clinically, acalculous and calculous cholecystitis are indistinguishable. Pain, vomiting, distention, and right-upper-quadrant tenderness and mass constitute most of the signs and symptoms (11). In several series, the mortality of acalculous disease far exceeds that of calculous disease. In one study it was reported to be twice that of disease associated with stones (11). Histologically, the degree of necrosis, gangrene, and perforation of the gallbladder wall is more frequent in AAC than in calculous disease.

It has been suggested that acalculous cholecystitis represents a more advanced stage of the same pathophysiologic process involved in calculous cholecystitis (11). The typical signs of acalculous cholecystitis may be very subtle in presentation or may be misinterpreted. The lack of high clinical and laboratory specificity in the diagnosis of cholecystitis may contribute to delay in making the proper diagnosis (1). In fact, only a high degree of suspicion may lead to the diagnosis. The treatment is the same in both types of cholecystitis, namely, surgical removal of the inflamed gallbladder. However, as the disease process progresses, so do postoperative complications and morbidity rates.

The purpose of this article is to review some of the current literature on acalculous cholecystitis, the settings in which the disease occurs, and the possible pathophysiologic mechanisms involved. Although AAC is still relatively rare, it is becoming more frequent in certain situations. A high degree of suspicion for AAC must be entertained, in light of the severity of the damage to the gallbladder. The risk of diagnostic error is far greater than that of a negative laparotomy (16).

AAC as a Postoperative Complication. Sporadic reports in the literature, of acute cholecystitis as a complication in the postoperative period, date back to 1906, when Kocker and Matti observed AAC after gastroduodenostomy (17). It was not until 1947 that Glenn discussed the increasing incidence of acute cholecystitis after surgical treatment of unrelated diseases (6). His study covered 17 patients from 1932 to 1946 who developed acute cholecystitis after surgical procedures unrelated to the biliary tract, including herniorrhaphies, appendectomies, drainage of leg abscesses, and nasal packings for epistaxis. Glenn speculated that it was fasting in the postoperative period that led to the development of AAC. Prolonged fasting caused the bile to become very viscous in consistency as it remained sequestered in the gallbladder. As the patient slowly resumed his regular diet, endogenous stimuli would cause the gallbladder to contract in an attempt to empty itself. The viscid bile, unable to traverse the narrow cystic duct with ease, would in effect cause a functional obstruction.

Womack and Haffner (18) reported that, experimentally, marked concentration and high viscosity of bile alone were sufficient to cause cholecystitis. This process would be further augmented by other features commonly found in the postoperative period. Fever, dehydration, and drugs such as morphine, meperidine, and codeine, which increase intraluminal pressures by prolonging contraction of the sphincter of Oddi, could play prominent roles in the process. As the gall-

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bladder failed to empty but continued to accumulate bile and mucin, distention would occur, which might eventually compromise the vascular circulation of the organ. Edema would then ensue, leading to diminished arterial supply, subsequent infarction, necrosis, and eventual perforation, if the process was allowed to continue. Early surgical intervention is recommended as the treatment of choice. Since the 1947 report, Glenn's observations have been confirmed by numerous other reports. Schwegman et al (8), Lindberg et al (4), and Howard and Delaney (3) have all noted the onset of cholecystitis to be temporally related to the resumption of oral intake after surgery.

AAC in Children. The development of acalculous disease has not been confined to the surgical patient. Reports of AAC in children, burn patients, septic patients, Vietnam veterans, and trauma victims can be found throughout the literature. Ternberg and Keating (15) reported on 74 cases of AAC occurring in children, in 60% of which the AAC developed as a complication of other primary illnesses such as systemic infections. In four cases there were congenital biliary tract lesions. Their conclusion was that AAC in children should be recognized as a complication of injury or illness such as burns or bacterial infection, similar to AAC in adults. They agree with Glenn and Becker's proposed mechanism of disease (10), whereby fever, dehydration, prolonged fasting, and prolonged ileus that accompany the primary illness are largely responsible for the biliary stasis and local damage that ensue. However, in children one must be alert for congenital lesions of the biliary tract, such as choledochal cysts or ductal atresia causing obstruction in the absence of stones.

AAC in Burn Patients. Munster et al (9) reported AAC developing as a complication in ten patients who had suffered extensive burns, on the average of more than 56.2% of their total body surface. Sepsis, multiple surgical procedures, and dehydration were cited as features common to all the patients with extensive thermal injury. The authors' conclusions centered around the severe dehydration found in burn patients, mainly due to insensible loss from burned surfaces. Although no precise mechanism of how the dehydrated state can lead to cholecystitis is defined, the authors believe that biliary stasis and sludging with superimposed bacterial seeding is the most likely sequence of events.

AAC in Trauma Patients. During the Vietnam War years, several reports appeared in the literature of soldiers who had sustained extensive trauma and who developed acalculous disease as a complication of their injuries. Lindberg et al (4) reported 12 and Weeder et al (5) reported six such cases in young men ranging in age from 19 to 30 years. The incidence of gallbladder disease in men of this age group in the general population is very low, so that this finding represented a significant observation.

The conclusion in the Lindberg article centered around two factors that were considered crucial. First, there was a group of patients in whom positive bile cultures were obtained. The organisms found could be traced to the bloodstream or the wounds sustained, suggesting that active infection was somehow involved in the progression of the disease. Second, each of the patients had received massive blood transfusions to replace the blood losses associated with fractures and amputations. This led the authors to suggest that hemolysis had produced large quantities of bilirubin, which could alter the composition of the bile and thus contribute to biliary sludging. Weeder et al came to similar conclusions in their article, which also mentioned dehydration, narcotic therapy, and fever as contributing to an increase in the bile viscosity.

DuPriest et al (1) reviewed the records of 11 patients who developed acalculous disease after trauma. Included in their review was a collection of 20 additional papers totaling 98 patients with acute cholecystitis. Eighty-seven percent had acalculous disease, whereas only 13% had stones. They found that the mortality rate of AAC was 35%, whereas that of calculous disease was only 14%. Among the possible pathogenetic mechanisms mentioned in their review of the literature was bile stasis, increased pigment load due to hemolysis, postoperative dehydration, and bacterial invasion of the gallbladder secondary to sepsis. However, they support the theory of Meissner (19), Thompson et al (20), and Golden et al (2), who proposed that hypotensive or shock states caused local vascular changes in the gallbladder leading to inflammation, infarction, and secondary bacterial invasion of the organ. Their proposal was that sympathetic activity during shock states caused a reflex vasoconstriction of the vessels supplying the gallbladder. This in turn would cause ischemia. Howard et al (21) found that sympathectomy had a protective effect in experimental canine acute cholecystitis.

AAC as a Consequence of Vascular Changes. In 1982 Glenn and Becker published results of a study comparing patients with acalculous vs calculous disease from 1932 to 1979 at the New York Hospital (10). They found the mortality rate of

AAC to be slightly more than twice that of calculous disease. They also found that the observed incidence of AAC had increased significantly between 1950 and 1979. Their histologic observations of the acalculous gallbladders emphasized the focal necrosis in the walls of the arteries and veins, as well as their infiltration by polymorphonuclear neutrophils. They claimed that early changes, such as focal injury of the endothelium or intima, led to the dissection of the endothelium by erythrocytes and ultimately thrombosis.

Glenn and Becker turned away from the traditional viewpoint of biliary stasis/sludging and instead emphasized the vascular changes leading to AAC. Their proposed mechanism centered around the activation of Hageman factor (XII) in the clotting cascade (22). It was felt that this was perhaps the initial step leading to thrombosis of the blood vessels of the gallbladder. This concept was alluded to earlier in hypotensive states, but the mechanism proposed then involved sympathetic vasoconstriction and arterial spasm rather than actual thrombosis. According to Glenn and Becker, in patients who have undergone trauma, exposure of their plasma to collagen activates Factor XII. In septic states, Gram-negative bacterial endotoxins can also activate the Hageman factor.

Lesions similar to those found in patients with acalculous disease have been produced in dogs and monkeys consequent to activation of Hageman-factor-dependent pathways by intravenous injection or inhalation of polyphenol or polyphenol-containing activators of these pathways, or by injection of bacterial endotoxins (10). Active Hageman factor fragments have been found in albumin preparations, and storage of plasma at 4°C causes conversion of prekallikrein to kallikrein, which in turn activates Hageman factor. Thus patients receiving blood and blood product transfusions could conceivably have activated clotting cascades.

Material resembling Factor XII with prekallikrein activation has been found in guinea pig skin. If similar material were found in human skin, it might be activated and released into the circulation consequent to major trauma to the skin, as in extensive burn injuries. Finally, many malignant cells synthesize plasminogen activator substances; their release into the circulation could result in the indirect activation of Factor XII.

Thus, trauma, sepsis, massive transfusions, burns, and cancer are all conditions under which an activated clotting cascade could occur. The question that must be addressed is why, if this mechanism is true, is there only a localized thrombotic event in the gallbladder and not a generalized thrombosis? Glenn and Becker speculate that since the gallbladder is embryologically derived from the duodenum, which contains CCK within its walls, the gallbladder may also have some type of kininogen within its walls. This kininogen may be cleaved by active components of the intrinsic pathway to yield vasoactive substances capable of augmenting local vasospasm and injury. Whether this vasospastic phenomenon alone is sufficient to cause cholecystitis or whether a secondary bacterial involvement is necessary is still a matter of controversy.

Finally, the authors cite several reasons for the increasing incidence of AAC over the last two decades, including the increasing age of patients, the more extensive and invasive therapeutic surgical procedures performed recently, and the prolonged survival of gravely ill patients owing to better resuscitative methods, which increases the chances of initiation of the disease.

AAC as a Complication of Hyperalimentation. The most recent reports in the literature concern the development of AAC in patients on long-term parenteral hyperalimentation. The first anecdotal report was by Anderson (12), followed by a second report by Petersen and Sheldon (13), who examined 435 patients at the San Francisco General Hospital between 1972 and 1977. In their study, 100 patients had documented acute cholecystitis; 17 were without stones. Eight of the seventeen had received total parenteral nutrition (TPN) ranging from 8 to 39 days, with a mean of 21 days. Their parenteral solution consisted of hypertonic glucose-crystalline amino acid infusions with fat emulsions given through a central venous catheter. All eight had either undergone laparotomy or had sustained some type of trauma, so that the design of the study was not entirely controlled for isolating the effect of hyperalimentation alone on the biliary tract. The conclusion of the study was that, again, biliary stasis and failure of the gallbladder to empty, owing to the lack of stimulation from the stomach and duodenum in the fasting state, were the most important factors contributing to the development of disease. They recommend that, periodically, small amounts of fat or CCK be administered, thus stimulating the gallbladder to contract in the absence of any oral intake. In this manner, any accumulation of bile would be avoided.

Another study of the effects of TPN on the biliary system was published in 1983 by Roslyn, et al (14), which focused not specifically on the relationship of TPN with acalculous disease, but with cholecystitis as a whole. They studied adult patients who had received prolonged courses of TPN (for a minimum of 3 months) at the UCLA Medical Center from 1976 through 1980. Of the 109 patients in their series, 25 developed cholecystitis after TPN (21 with stones and four without stones). Of the 109 patients, 79 had some preexisting disorder of the ileum, such as Crohn's disease or a previous bowel resection, and 30 had a nonileal disorder. Because of the known predisposition that patients with ileal disorders have in developing gallbladder pathology, they were considered separately in this study. The parenteral solution given was similar to that described by Petersen and Sheldon (13).

Roslyn et al compared their results to those reported by Heaton and Read, which they used as a control group. The patients were similarly defined in both studies except that those in the Heaton and Read study had not received TPN. When compared, it was found that TPN patients with ileal disorders had a 29% incidence of gallbladder disease, whereas the non-TPN patients with ileal disease had a 25% incidence. Because of the retrospective nature of the study, the authors claim that their figures would probably be slightly lower than would be expected. They could not provide for entities such as asymptomatic stones, which would be recorded as no disease of the gallbladder, when there was in fact pathology present. This difference (29% vs 25%) in the patient groups was considered by the authors to be significant. Furthermore, it was found that 33% of TPN patients developed cholecystitis within 5 years of ileal resection, as compared to 13% of similarly defined patients who were not on TPN, again suggesting that TPN is a significant factor in promoting gallbladder disease.

The authors went on to compare TPN and non-TPN groups for various risk factors and their relationship to gallbladder disease. The factors they found to be significant were fasting for greater than 80% of the time spent on TPN, the chronic usage of narcotics, the chronic usage of anticholinergics, and Crohn's involvement of the terminal ileum, all of which caused an increase in the incidence of developing cholecystitis. Thus being on TPN increased the risk of developing gallbladder disease, but a variety of additional factors may augment that risk. These findings, especially the correlation of time spent in the fasting state with development of the disease, suggest that perhaps biliary stasis and biliary concentration are indeed the basic underlying mechanisms in acute cholecystitis in patients on TPN. Whether the biliary stasis was responsible for stone formation, which then led to the cholecystitis, or whether the stasis itself was sufficient to cause the cholecystitis is not clear. The authors conclude that further work needs to be done before a definitive conclusion can be reached.

Summary. In summary, there appears to be an abundance of case reports of acute acalculous cholecystitis appearing in a wide variety of clinical settings. That AAC does occur is now widely accepted and recognized, but why it occurs is still speculative. Diagnosis, or suspicion of the entity is necessary because early treatment is essential. How a single common pathophysiologic mechanism can account for development of the disease under such a wide spectrum of situations, or whether there is a single mechanism, is the center of the issue.

There are two main schools of thought. One centers around conditions that cause bile to lie stagnant in the gallbladder and thus lead to cholecystitis. The high concentrations of bile being directly toxic, the overdistention of the gallbladder leading to vascular compromise, and the stagnant bile acting as an excellent milieu for secondary bacterial invasion, along with the proposal that the viscous bile acts as a functional obstruction to outflow from the gallbladder, have all been suggested as possible pathways.

The second theory involves the vascular supply to the organ as the direct cause of the disease process. There appear to be factors common to most cases of AAC that can cause activation of the Hageman factor and thereby set in motion the clotting cascade. This causes selective thrombosis of the vessels supplying the gallbladder, setting up an ischemic state, leading to necrosis, secondary bacterial involvement, and eventual infection and perforation through the gallbladder wall.

Both theories have merit and are plausible, but neither can explain the development of the disease in all situations. The two schools of thought are not mutually exclusive, however; it is quite likely that what occurs in vivo involves a combination of the two.

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The Aberrant Puberty Hypothesis of Polycystic Ovarian Disease: A Review

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A syndrome consisting of bilaterally enlarged polyfollicular ovaries in association with infertility, menstrual dysfunction, and hirsutism was originally described in 1935 (1). A spectrum of varying signs and symptoms with or without ovarian enlargement, hirsutism, anovulation, or infertility was subsequently reported (2, 3). The term "polycystic ovarian disease" (PCOD) is applied to a wide clinical array of ovarian, adrenal, and mixed ovarian and adrenal disorders which eventually culminate in polyfollicular ovaries in various stages of growth and atresia. A thickened tunica albuginea and increased thecal luteinization are frequently but not invariably present in this disorder (4). The frequency of PCOD in the general population has been estimated to be between 3.5% and 7.0% (5, 6).

Despite the wide variation in clinical findings, PCOD is usually associated with obesity, hyperandrogenemia, increased extraglandular conversion of androgens to estrogens, and a characteristic inappropriately elevated ratio of luteinizing hormone (LH) to follicle-stimulating hormone (FSH) (2, 3). Inappropriate gonadotropin secretion, that is, increased LH/FSH ratio, occurs in approximately 70% of patients with PCOD. Repeated pooled sampling of serum LH and FSH for several cycles in patients suspected of having PCOD may further increase the reported frequency of this secretion, an important feature of PCOD.

We have previously hypothesized that the etiology of the predominantly LH-dependent type of ovarian hyperandrogenemic PCOD is due to an aberrant puberty (7). We have also suggested that PCOD may be represented by two distinct stages: a causative central-nervous-system generator stage and a resultant effector stage, manifested by a series of events involving the ovaries and brain.

Inappropriate Gonadotropin Secretion. Inappropriate gonadotropin secretion is characteristic of the generator stage in PCOD. It may result from a pubertal decreased set point (increased hypothalamohypophyseal sensitivity) to the negative feedback effect of ovarian steroids on FSH as well as to the positive feedback on LH release. The perpetuation of inappropriate gonadotropin secretion in the effector stage of PCOD is accomplished by a series of events (Fig. 1). Decreased circulating levels of FSH from the generator stage result in reduced intrafollicular FSH concentration, androgenic follicular microenvironment, decreased granulosa cell population, and diminished aromatase activity (8). This is associated with suboptimal conversion of LH-induced thecal and stromal androgens into estrogens. Chronic intrafollicular hyperandrogenemia enhances follicular atresia, whereas LH-dependent ovarian androgens (androstenedione > testosterone) elicit further inhibin production from granulosa cells of the more viable follicles (9).

FSH release is preferentially inhibited by inhibin and estrone (10, 11); estrone is the principal product of extraglandular aromatization of androstenedione. Hyperandrogenemia results in reduced sex-hormone binding globulin (SHBG) furnishing increased levels of free serum testosterone, estrone, and estradiol (12). Whereas estrone (E_1) preferentially inhibits FSH secretion, estradiol (E_2) generally favors LH release by increasing pituitary gland sensitivity to gonadotropin-releasing hormone (GnRH).

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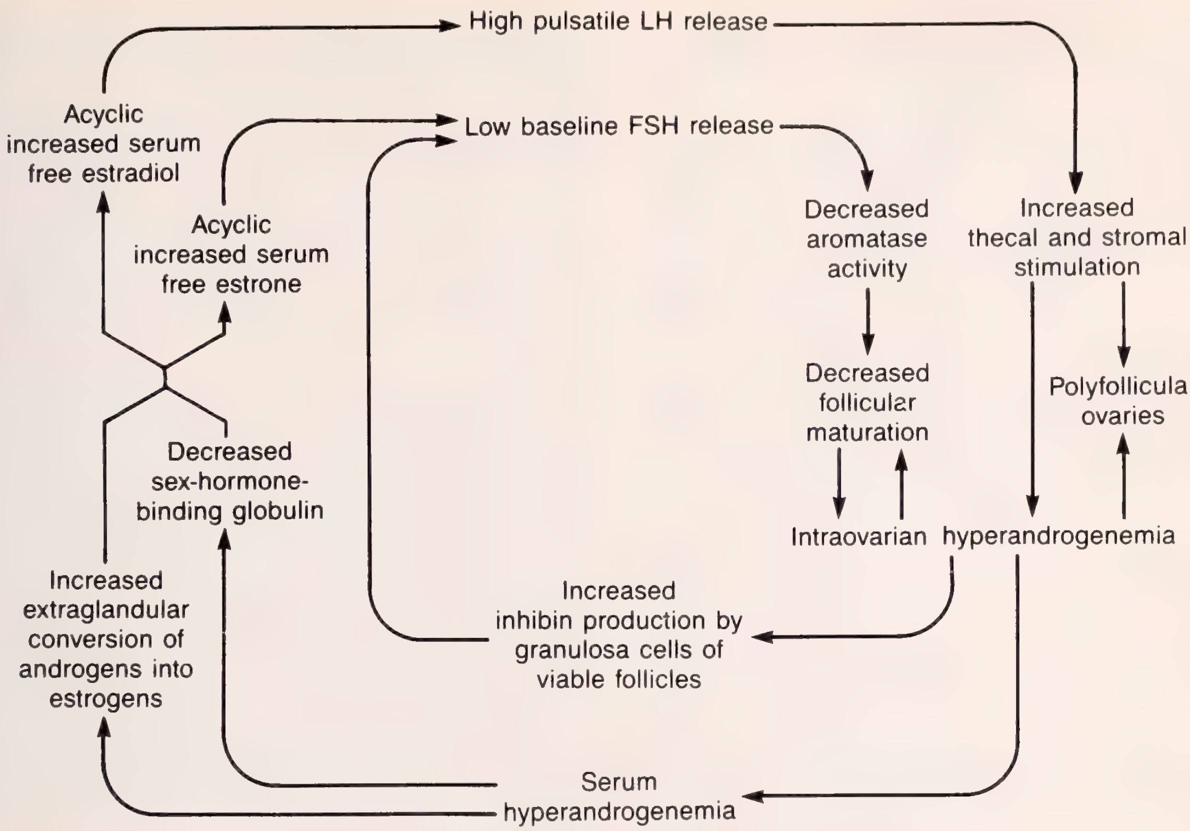
High pulsatile LH release
Low baseline FSH release
Acyclic increased serum free estradiol
Acyclic increased serum free estrone
Decreased sex-hormone-binding globulin
Increased extraglandular conversion of androgens into estrogens
Decreased aromatase activity
Increased thecal and stromal stimulation
Decreased follicular maturation
Polyfollicular ovaries
Intraovarian hyperandrogenemia
Increased inhibin production by granulosa cells of viable follicles
Serum hyperandrogenemia

Fig. 1. Effector stage in polycystic ovarian disease.

In this manner, ovarian hyperandrogenism perpetuates inappropriate gonadotropin secretion. The purpose of this report is to present a more detailed analysis of this model, which hypothesizes possible pubertal events that set the stage for the emergence of polyfollicular or "polycystic" ovaries.

Theories of Pubertal Onset

Puberty is the period in human development during which sexual reproduction first becomes possible. It is further characterized by accelerated linear growth, appearance of secondary sexual characteristics, and psychosexual changes. The hypothalamohypophyseal events that govern these changes are complex and at present unclear.

Hypothalamic Gonadostat. Grumbach et al (13, 14) have suggested that the equilibrium between hypothalamic drive and ovarian steroid feedback is determined by a hypothalamic gonadostat. According to this model, human chorionic gonadotropin stimulates the fetal ovaries to produce androgens and estrogens early in gestation. By 80 days, fetal pituitary gland LH and FSH appear and also influence the ovaries (13). By 100–150 days, hypothalamic GnRH stimulates additional hypophyseal LH and FSH and thus ovarian sex steroids (13). By 150 days, a hypothalamic set point to sensitivity of steroidal feedback on GnRH secretion develops. ("Set point" is a term describing the threshold at which the hypothalamus responds to positive or negative steroidal feedback. A high set point therefore corresponds to a low sensitivity to feedback, and vice versa.)

Gonadotropins and ovarian secretions are low at term and remain so until the second postnatal week, when ovarian secretion rises, and then, by age six months, both gonadotropins and ovarian steroids gradually decline. A high sensitivity to negative steroidal feedback persists until early puberty, when the set point rises, thereby decreasing the impact of the negative feedback on the hypothalamus (13). This derepresses GnRH secretion. At late puberty, the set point for positive steroidal feedback decreases, facilitating increased sensitivity to positive feedback (13). The latter facilitates pulsatile LH, promoting the first LH surge and ovulation.

Although accurate in its attempt to explain greater hypothalamic sensitivity to negative feedback of estradiol in prepubertal, as compared to adult, females, the gonadostat model fails to describe the government of the gonadostat itself, that is, what is actually responsible for the neuro-

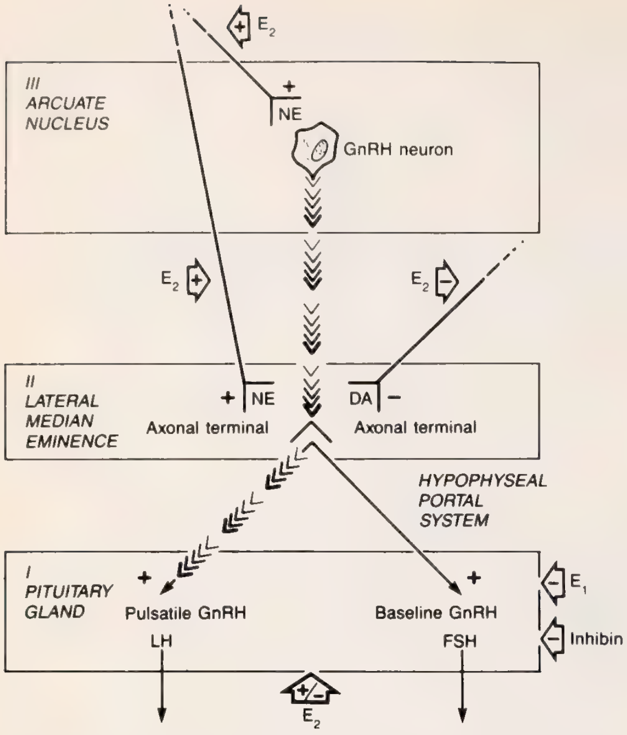

Fig. 2. Three neuroendocrine loci controlling gonadotropin secretion. NE, norepinephrine; DA, dopamine.

chemical nature, timing, and neuroanatomic loci for set-point changes.

Brain as Control. Ruf (15) has suggested that set-point changes during resetting of the hypothalamic gonadostat occur through growth and functional maturation of steroid-sensitive adrenergic systems in the brain. During the prepubertal period, an equilibrium is established between low hypothalamic drive and low ovarian steroid secretion (15). Although ovarian secretion rises during early puberty, this secretion is outpaced by an increase in body distribution volume during accelerated body growth. This results in decreased steroid concentrations at brain receptor sites (15).

Thus, the presence of a quantitatively unchanged hypothalamic drive, which occurs concomitantly with less ovarian negative feedback, suggests an apparent decreased hypothalamic sensitivity (high set point) to negative steroid feedback. In the latter stages of puberty an equilibrium is reestablished by augmenting hypothalamic drive via an increased terminal arborization of stimulatory hypothalamic adrenergic neurons (15). This produces a new equilibrium between high ovarian steroid output and high hypothalamic GnRH drive, leading to the first LH surge.

It is apparent from this model that control mechanisms of the adolescent growth spurt as well as adrenergic hypothalamic proliferation are important to the timing of pubertal onset.

The initiation of puberty is dependent on pulsatile LH and LH surges (16). Tonic GnRH presentation would regulate hypophyseal GnRH receptors until LH secretion is extinguished (17). Pulsatile GnRH, however, permits regeneration of GnRH receptors and adequate resultant LH pulses (17). The appearance of an LH surge (a single pulse with great amplitude) probably results from both increased positive steroidal feedback and decreased negative steroidal feedback.

Aberrant Puberty in Polycystic Ovarian Disease

It has recently been reported that pubertal girls with PCOD have desynchronized LH surges (18). This implicates the pubertal central nervous system as an important participant, and perhaps the initiator, of PCOD pathophysiology. We have hypothesized that inappropriate gonadotropin secretion is generated by a decrease in set point to both negative feedback on FSH during early puberty and positive feedback on LH during late puberty (7). Events that precipitate these set-point changes may occur at three neuroendocrine loci of the hypothalamohypophyseal system: the anterior pituitary gland, the median eminence, and the arcuate nucleus (Fig. 2).

Anterior Pituitary Gland

Gonadotropin Release. Estradiol (E_2) exerts both negative and positive feedback on gonadotrope secretion, depending on its concentration and duration of effect. Chronically elevated E_2 levels increase the rapidly releasable LH pool (19). Estrone (E_1) and inhibin appear to preferentially inhibit FSH secretion (10, 11). Therefore, set-point variations in secretory rates of FSH and LH may result from gonadotrope membrane or exocytotic dysfunction.

Gonadotrope Sensitivity to Estrogen and GnRH. The set point of the anterior pituitary gland to hypothalamic and ovarian messages during puberty is determined by alterations in (a) gonadotrope number, (b) estrogen and GnRH receptor binding affinity, (c) ability to regulate GnRH receptors in response to pulsatile GnRH, and (d) intracellular transduction of receptor-mediated information into LH or FSH synthesis.

Median Eminence

The external palisade of the median eminence can be divided into medial and lateral zones. Tuberoinfundibular dopamine terminals in the medial median eminence secrete dopamine directly into the portal vasculature, which inhibits lactotrope secretion of prolactin (20). In the lateral median eminence, however, dopamine terminals are located near GnRH terminals (21). Such dopamine diffuses through the tissue and inhibits GnRH release (21). In the lateral median eminence are norepinephrine-containing terminals that are also located near GnRH terminals; norepinephrine stimulates GnRH secretion and thus triggers the LH surge (22).

The multifaceted effects of sex steroids on lateral median eminence catecholaminergic neurons (dopamine and norepinephrine) are currently unclear. The effects are probably dependent on the concentration and cyclicity of serum steroid levels. Elevated acyclic estrogen levels may indirectly stimulate GnRH secretion by inhibiting dopaminergic neurotransmission (23) or enhancing noradrenergic neurotransmission (Fig. 2). The intrinsic ontogeny and plasticity of catecholaminergic systems is genetically determined and influenced by the perinatal steroidal environment in the brain (24). During prepuberty, the capacity of dopamine and norepinephrine arborization in the lateral median eminence determines the eventual equilibrium between hypothalamic GnRH drive and set point to ovarian steroidal feedback.

Arcuate Nucleus

The major site of GnRH synthesis is believed to be the arcuate nucleus. Arcuate nucleus turnover of norepinephrine increases during late proestrus in rats (22). This suggests that norepinephrine may directly affect GnRH neuron cell bodies in triggering the LH surge (22). Similarly, the high estrogen levels at this time might facilitate norepinephrine stimulation, thus augmenting GnRH pulse frequency and amplitude. The set point at the level of the arcuate nucleus is therefore dependent on sex steroid sensitivity of norepinephrine neurons and the development of competent norepinephrine neuronal synapses with GnRH neurons.

Decreases in Set Point

During puberty, normal set-point changes to steroidal feedback include early, gradually increasing sensitivity to estrogen by lateral median eminence dopamine neurons (derepressing GnRH release), and subsequent increasing sensitivity to estrogen by arcuate nucleus norepinephrine neurons (stimulating LH pulses and surges).

In PCOD, however, we hypothesize greater pubertal catecholaminergic arborization (dopamine and norepinephrine), which has two effects. First, if more lateral median eminence dopamine neurons are present, normal early pubertal amounts of estrogen will inhibit less dopamine. More dopamine is then available to inhibit baseline GnRH release; the result is diminished FSH secretion. Second, when relatively more norepinephrine neurons impinge on arcuate nucleus GnRH neurons, more norepinephrine is available to respond to the proestrus rise in estrogen. This results in a greater stimulation of GnRH pulse frequency and amplitude. Furthermore, since LH secretion is primarily dependent on GnRH pulses, LH pulses will be enhanced. A low baseline FSH level and a high pulsatile LH secretion combine to produce inappropriate gonadotropin secretion (Table). Alterations of set point in the anterior pituitary gland, as well as the presence of metabolic messengers, modify the net effect, allowing for different degrees of inappropriate gonadotropin secretion.

TABLE

Neuroendocrine Mechanisms of Steroid Feedback on GnRH Secretion

	Negative feedback baseline GnRH	Positive feedback pulsatile GnRH
Factors		
Neuroanatomic locus	lME	Arcuate nucleus
Catecholamine	DA	NE
Prepubertal hypothalamic drive	Low tonic FSH	Nonpulsatile LH
Normal Puberty		
Set Point	Increases, early	Decreases, late
Effect	Increased baseline FSH	Increased LH pulse amplitude, frequency
Mechanism	Increased sensitivity to inhibitory effects of E_2	Increased sensitivity to stimulatory effects of E_2
PCOD		
Set point	Attenuated increase, early puberty	Enhanced decrease, late puberty
Effect	Decreased FSH	Exaggerated LH pulses
Mechanism	Increased lME DA proliferation in prepuberty	Increased arcuate nucleus NE proliferation in prepuberty

lME, lateral median eminence; DA, dopamine; NE, norepinephrine.

The effector stage of PCOD is marked by ovarian hyperandrogenism, which leads to hirsutism, polyfollicular ovaries, chronic anovulation, dysfunctional bleeding, and an increased incidence of endometrial carcinoma (3). Unopposed 17-beta-E_2 stimulates adipocyte replication and may therefore increase a tendency toward obesity (25). Adipose tissue also contributes to the increased peripheral aromatization of androgens into acyclic estrogens. Elevated brain estrogen levels also inhibit dopaminergic tone in the medial median eminence, producing lactotrope hyperplasia and hyperprolactinemia. In some cases accelerated growth of an occult pituitary adenoma may follow (26, 27). Hyperprolactinemia occurs in approximately 20%–30% of patients with PCOD (2, 28).

Summary

The etiology of LH-dependent ovarian PCOD which occurs without significant adrenal hyperandrogenism is postulated to be an aberrant puberty. We hypothesize that central nervous system alterations in set point to steroidal feedback occur at three neuroendocrine loci: anterior pituitary gland, median eminence, and arcuate nucleus. This is brought about by two pubertal events: increased proliferation of lateral median eminence estrogen-sensitive dopamine neurons, which diminish baseline GnRH and FSH secretion rates, and increased proliferation of arcuate nucleus estrogen-sensitive norepinephrine neurons, which enhance pulsatile GnRH and LH secretion. Such catecholaminergic changes in puberty produce a state of inappropriate gonadotropin secretion that generates PCOD. Therapeutic goals can be better assessed in PCOD by an improved understanding of the specific temporal and physical neuroendocrine mechanisms involved.

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Enteropancreatic Reflexes 3: The Effect of Ileal Distention on Pancreatic Exocrine Secretion

ALBERTO SALINAS, M.D., EHUD KLEIN, M.D., ANDREW T. TRIEBLING, M.D., LEWIS TOTH, PH.D., AND DAVID A. DREILING, M.D.

Abstract

There is good evidence for the existence of gastropancreatic reflexes. We have previously presented data showing decreased exocrine pancreatic secretion during jejunal distention. This study was designed to determine whether such changes could originate in the terminal ileum. Nine dogs were prepared with Thomas cannulas in the duodenum and ileal Thiry loops 5 cm proximal to the ileocecal valve. Pancreatic secretion was collected by direct cannulation of the pancreatic duct and analyzed for volume, bicarbonate, and protein (enzyme). The effect of distention of the ileal loop was studied with the animals in the conscious state under basal and stimulated (secretion 0.03 units/kg/min) conditions. In the basal state, ileal distention resulted in a reduction of 60% in volume of secretion ($p < 0.01$). In the secretin-stimulated experiments there was a consistent inhibition of secretion of 35% ($p < 0.01$). A concomitant reduction in bicarbonate concentration was also demonstrated (15%—$p < 0.01$). Enzyme concentrations were not significantly affected. The inhibitory effect of ileal distention could be blocked by atropine (standard dose of 1 mg intravenously). Adrenergic blockers (propranolol, 0.1 mg/kg, and phentolamine, 0.25 mg/kg, intravenously) did not block this inhibitory response. This study suggests the existence of atropine-sensitive enteropancreatic reflexes, arising in the terminal ileum and exerting an inhibitory effect on pancreatic exocrine secretion.

White and Magee (1, 2) demonstrated the existence of gastropancreatic reflexes inducing increased pancreatic secretion following gastric distention. Other authors (3) have not been able to establish any relationship between mechanical distention of the duodenum and pancreatic secretion. We recently presented (4) data that demonstrate the existence of enteropancreatic reflexes in which distention of the jejunum inhibited exocrine pancreatic secretion mediated through a cholinergic pathway.

The present study was undertaken to determine whether enteropancreatic reflexes could be elicited by distention of the terminal ileum.

Materials and Methods

Six adult mongrel dogs, weighing 15–20 kg, were prepared with Thomas cannulas (5) placed in the duodenum after preliminary ligation of the accessory pancreatic duct. At the same operation a 12-cm segment of ileum taken 5 cm proximal to the ileocecal valve was fashioned into a Thiry loop with the proximal end closed, left intraperitoneally, and the distal opening exteriorized through the abdominal wall. Secretory tests were begun two weeks after surgery. The animals were trained to stand quietly without sedation. Each observation was carried out with the animals in the conscious state, deprived of food but not water for at least 24 hr. Animals were studied no more than twice a week and never on consecutive days. A continuous intravenous infusion of 0.45% of sodium chloride was maintained

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AS and EK were Alfred and Florence Gross Research Fellows. LT was supported by the Alfred and Florence Gross Research Fund. DAD is Alfred and Florence Gross Professor of Surgery.

throughout each experiment at a rate of 20 cc/hr to allow for fluid losses.

The effect of distention of the ileal loop on exocrine pancreatic secretion was studied under basal and secretin-stimulated conditions. Pancreatic secretion was collected by direct cannulation of the main pancreatic duct under direct vision through the Thomas cannula at 30-min intervals in basal condition and 5-min intervals in the secretin-stimulated experiments. Secretin (The Boots Company Ltd.), 0.03 unit/kg/min, was administered intravenously. When a plateau was reached, distention of the ileum was produced by inserting a Foley catheter into the Thiry loop through the opening in the abdominal wall. The balloon was inflated with 25 cc of air. Distention of the loop with this amount of air was checked fluoroscopically in each dog. In no case did distention appear to cause pain. After two to three collections during distention of the balloon, the balloon was deflated and new samples were taken immediately thereafter.

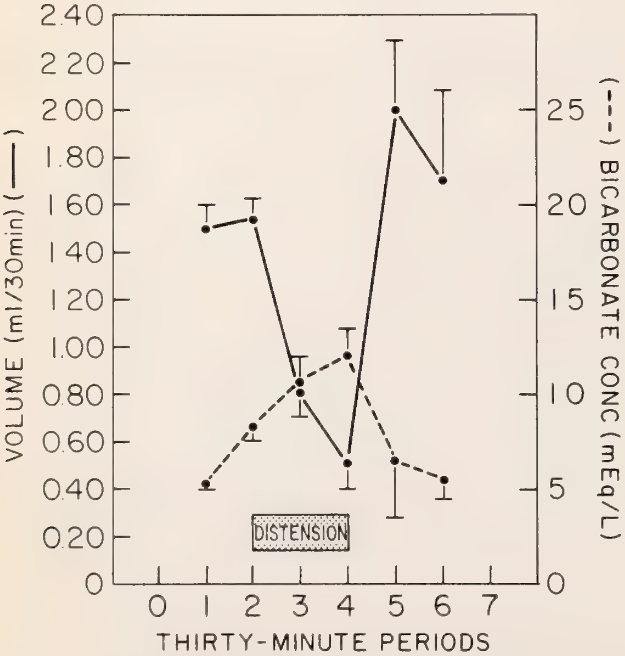
In another group of experiments (three dogs), secretion was studied before and after ileal distention in animals previously treated with atropine (standard dosage of 1 mg intravenously), a β-adrenergic blocker (propranolol, 0.125 mg/kg intravenously), or an α-adrenergic blocker (phentolamine, 0.25 mg/kg intravenously). In each experiment there was a 10-min interval between the administration of each drug and the distention of the ileal loop. Each pancreatic sample collected was analyzed individually for volume, bicarbonate, and protein concentration, the latter serving as a measure of enzyme secretion. Bicarbonate was measured with a Beckman Cl/CO_2 analyzer. Protein determinations were performed as described by Preshaw and associates (3). Statistical significance was determined by Student's *t* test on the basis of paired observations between control values and values following jejunal distention with and without the drugs used on each dog.

Results

Basal Secretion. Ileal distention resulted in a significant reduction (60%) in flow from 1.6 ± 0.28 to 0.5 ± 0.28 (<0.01). After release of the balloon, the flow returned to basal levels (Fig. 1). Mean bicarbonate concentration increased from 6 ± 0.8 mEq/L to 11 ± 2.0 mEq/L, a change not statistically significant.

Secretin Stimulation. Table 1 presents the volume response to a submaximal dose of secretin (0.03 unit/kg/min) before and immediately after ileal distention. Flow was reduced 35% ($p < 0.05$). The bicarbonate concentration (Table 2) decreased 15% ($p < 0.01$). Bicarbonate output, however, was reduced by 40% ($p < 0.01$). Following release of distention, both parameters returned to normal levels (Fig. 2).

The secretory rate following atropinization was not affected by ileum distention (Fig. 3). An atropine dose of 1 mg intravenously appears to block the inhibitory effect of ileal distention on both flow (Table 1), bicarbonate concentration (Table 1), and bicarbonate output (Table 2).



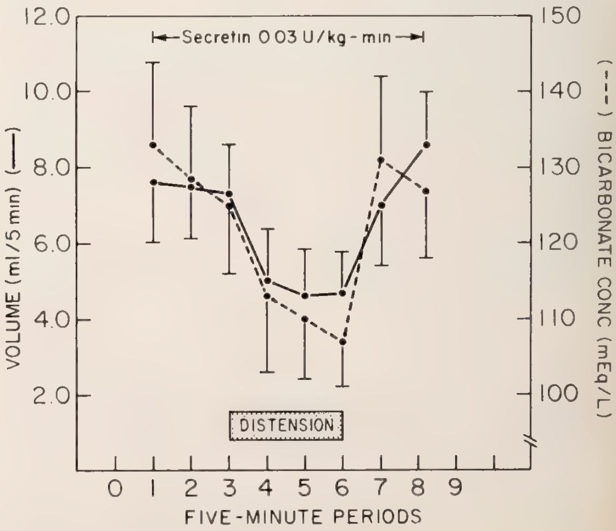


Fig. 1. Left Effect of ileal distention on basal (unstimulated) pancreatic secretion.

Fig. 2. Right Effect of ileal distention on secretin-stimulated pancreatic flow and bicarbonate concentration.

TABLE 1

The Effects of Atropine, Propranolol, and Phentolamine on Pancreatic Flow before and after Ileal Distention (Means ± SE)

	No. exp.	Control (ml/5 min)	Distention (ml/5 min)	Change
Secretin	24	7.5 ± 0.87[a]	4.9 ± 0.77*	−35%
Secretin + atropine IV	15	2.8 ± 0.39[b]	3.3 ± 0.29	+19%
Secretin + propranolol IV	15	4.6 ± 0.23[b]	2.4 ± 0.17**	−48%
Secretin + phentolamine IV	10	4.7 ± 0.18[b]	2.0 ± 0.25***	−57%

[a] Average of six dogs.

[b] Average of dogs.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.01$.

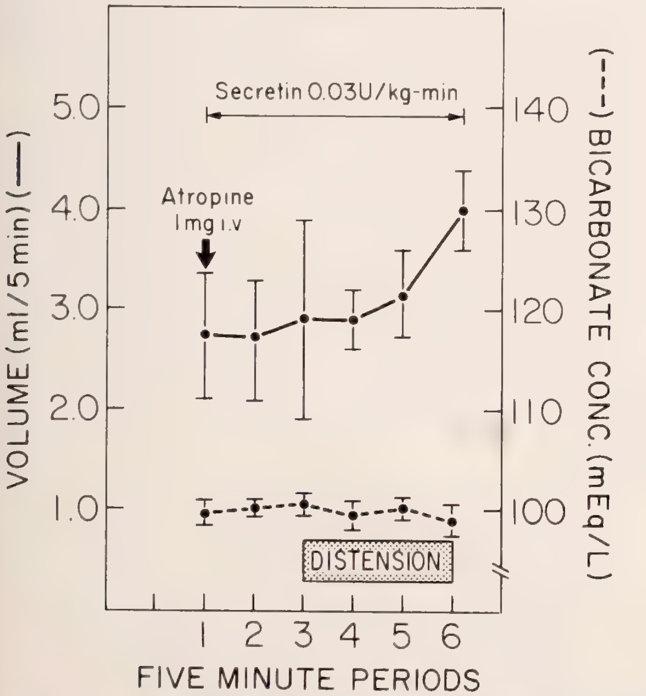
Several attempts to block the ileopancreatic reflex with topical lidocaine were not successful. Intravenous propranolol and phentolamine did not block the inhibitory effect of ileal distension (Fig. 4). A similar pancreatic response was obtained with and without these drugs, 35% of decrease in volume (Table 1) and 14%–9% decrease in bicarbonate concentration (Table 2).

The effect of atropine, propranolol, and phentolamine on the enzyme response to ileal distention is given in Table 3.

Discussion

These results demonstrate that mechanical distention of the ileum inhibits pancreatic exocrine secretion. Flow and bicarbonate concentration were significantly reduced. The enzyme output decrease appeared related to reduction of volume. In the phentolamine experiments, the reduction was statistically significant, possibly owing to activity of the drug itself as reported by Demol and Sarles (6). The pancreatic inhibition in all experiments was seen immediately after the ileal distention, that is, within 5–10 min. Control rates of secretion were reached 5–10 min after relaxation of the balloon.

Several studies have shown an inhibitory influence of the distal small bowel and colon on canine pancreatic secretion: infusion of fatty acids



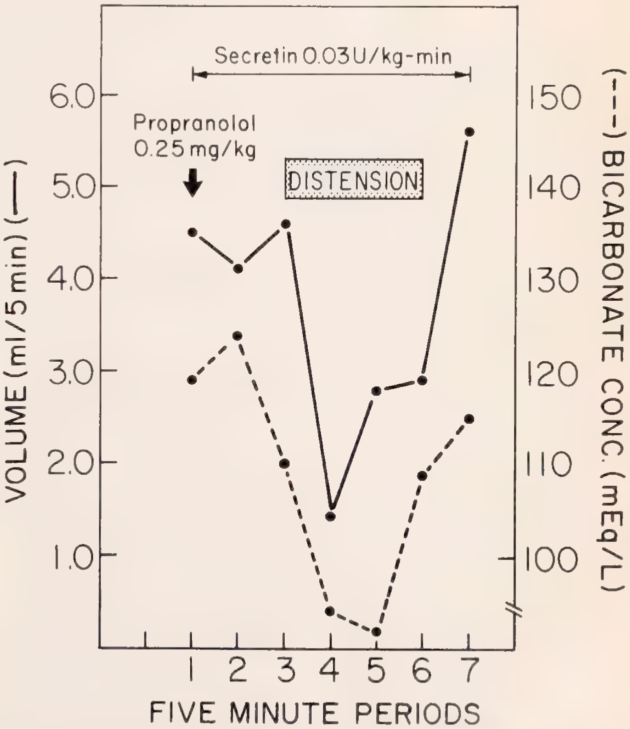


Fig. 3. Left Effect of intravenous atropine on inhibitory action of ileal distention on secretin-stimulated pancreatic flow and bicarbonate concentration.

Fig. 4. Right Effect of propranolol on inhibitory action of ileal distention on secretin-stimulated pancreatic flow and bicarbonate concentration.

TABLE 2

The Effects of Atropine, Propranolol, and Phentolamine on Pancreatic Bicarbonate Concentration and Output (Means ± SE)

	No. exp.	Bicarbonate control	Concentration distention	Change (mEq L)	Bicarbonate control	Output distention	Change (mEq/5 min)
Secretin	24	129 ± 6.0	111 ± 4.8*	+15%	0.94 ± 0.1	0.56 ± 0.09*	−40%
Secretin + atropine IV	15	99 ± 3.9	98 ± 4.7	−1.9%	0.25 ± 0.03	0.31 ± 0.03	+24%
Secretin + propranolol IV	15	113 ± 5.8	103 ± 2.7	−9%**	0.51 ± 0.03	0.24 ± 0.02*	−53.9%
Secretin + phentolamine IV	10	111 ± 2.36	101 ± 4.1***	−9%	0.51 ± 0.02	0.21 ± 0.02*	−59.8%

* $p < 0.01$.
** $p < 0.1$.
*** $p < 0.05$.

TABLE 3

The Effects of Atropine, Propranolol, and Phentolamine on Pancreatic Protein Secretion before and after Ileal Distention (Means ± SE)

	No. exp.	Protein control	Concentration distention	Change (mg ml)	Protein control	Output distention	Change (mg/5 min)
Secretin	6	6.6 ± 0.97	4.6 ± 0.74	−29%	45.7 ± 6.22	18.3 ± 4.5	−60%
Secretin + atropine IV	6	4.8 ± 1.02	5.5 ± 1.16	+14%	17.7 ± 7.61	20.6 ± 7.21	+16%
Secretin + propranolol IV	5	9.3 ± 1.07	9.6 ± 0.61	+3%	48.3 ± 8.57	22.7 ± 2.87	−53%
Secretin + phentolamine IV	5	8.5 ± 1.13	4.8 ± 0.48	−43%*	40.4 ± 4.88	12.0 ± 1.66	−70%

* $p < 0.01$.

intraluminally in experimental animals (7, 8). Similar results have been achieved in humans as well (5). The possibility of a hormonal basis to explain this phenomenon has been suggested by some authors based on experiments done in cats with a denervated pancreas (9) as well as on cross-circulation studies in rats (7). Among the epithelial peptides known, pancreatic polypeptide (pp) could be the only one associated with these observations. This polypeptide inhibits the secretion of pancreatic juice and particularly of pancreatic enzyme both in basal state and when secretion is stimulated by an infusion of cholecystokinin-pancreatozymin and secretin (10). Pp is detectable in the jejunum, but in a much lower concentration than in the pancreas (11). There is also good evidence of an intestinal release mechanism of pp; that is, an atropine-sensitive enteropancreatic reflex, to explain the biphasic response of pp to food (12, 13). Moreover, the release of pp is blocked by truncal vagotomy (14). Previous studies have shown the existence of gastro- and pyloropancreatic reflexes (1, 15), vagally mediated. In fact, long vasovagal reflexes are believed to be responsible for the majority of the exocrine pancreatic response to intraluminal amino acids and lipids (16). In this study, systemic atropine blocked the inhibitory effect of ileal distention, suggesting a cholinergic mediation similar to release of pancreatic polypeptide. However, the pancreatic response obtained after ileal distention is different from that obtained with pancreatic polypeptide (10). Catecholamines do not appear to play any role in these studies, since adrenergic blocking agents did not affect the pancreatic inhibitory action of ileal distention. The participation of hormonal mediators in these phenomena requires future investigation.

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Enteropancreatic Reflexes 4: The Effect of Colonic Distention on Pancreatic Exocrine Secretion

MOSHE KOLLER, M.D., ANDREW T. TRIEBLING, M.D., LEWIS TOTH, PH.D. AND DAVID A. DREILING, M.D.

Abstract

The effect of right and left colon distention was studied in a group of dogs with chronic pancreatic and gastric fistulas. Distention of the left colon did not significantly affect pancreatic secretion. Inflation of a balloon in an isolated loop of the right colon markedly decreased pancreatic secretion. Volume was decreased by 44%, bicarbonate concentration by 8%, and bicarbonate output by 48%. The decrease in protein concentration was 65% and in protein output 87%. Intravenous atropine blocked this effect; administration of α and β blockers did not. It is suggested that this phenomenon, distention of the right colon, affects pancreatic secretion by an enteropancreatic reflex, mediated by a neural, presumably vagal, or a neurohormonal mechanism.

Pancreatic exocrine secretion is responsive to sympathetic and parasympathetic activity, to a large number of gastrointestinal hormones, and to various locally released and locally acting neurotransmitters as well as to local and distant enteropancreatic reflex arcs. Distention of the body of the stomach in the dog produces an increase in pancreatic enzyme secretion that is blocked by atropine and by vagotomy, but not by gastric acidification, suggesting direct neural input rather than gastrin-mediated stimulation (1, 2). Gastropancreatic reflexes were demonstrated by White et al following different surgical procedures on the stomach (3). The role of the small bowel in hormonal regulation of pancreatic secretion is well established (4). We have demonstrated the existence of enteropancreatic reflexes following distention of the jejunum (5) and ileum (preceding article). Such distention resulted in inhibition of basal as well as secretin-stimulated pancreatic secretion (5, 6). The present study reports the effect of distention of the right and left colon on pancreatic exocrine secretion.

Materials and Methods

Four adult mongrel dogs weighing 16–18 kg were prepared with a Thomas cannula in the duodenum opposite the major pancreatic duct after preliminary ligation of the accessory duct. A gastric cannula was positioned in the most dependent part of the stomach to divert gastric secretion from the duodenum during subsequent experiments. At the same time, a segment of 12 cm of the right colon was isolated with its distal end closed and left intraperitoneally and the proximal end exteriorized through the abdominal wall.

Stage I. A Foley catheter was inserted through the rectum into the left colon for a distance of 10–15 cm. A continuous intravenous infusion of 0.45% sodium chloride was maintained throughout each experiment at a rate of 50 cc/hr to allow for fluid losses. The effect of distention of the left colon was studied following secretin stimulation. Pancreatic secretion was collected by di-

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TABLE 1
Left Colon Distention with Secretin Stimulation (Secretin 0.03 unit/kg/min)

		Control	Distention	Change
Volume	ml/5 min	4.5 ± 1.7	4.6 ± 1.9	+2.2%*
Bicarbonate	m Eq/liter	121 ±	127 ±	+4.9%*
Protein	mg/ml	9.9 ± 1.3	9.9	−0.8%*

* No significant statistical difference.

TABLE 2
Effect of Right-Colon Distention on Exocrine Pancreatic Secretion

	No. exp.	Control	Volume (ml/5 min) distention	Change
Secretin	11	5.2 ± 0.3	2.9 ± 0.25	−44%*
Secretin + atropine	10	2.5 ± 0.3	2.7 ± 0.3	+6%
Secretin + phentolamine	6	5.6 ± 0.6	3 ± 0.4	−46%*
Secretin + propranolol	9	5.1 ± 0.5	2.2 ± 0.2	−56%*

* $p < 0.01$.

rect cannulation through the Thomas cannula at 5-min intervals. Secretin (Kabi Vitrum AB), in doses of 0.03 unit/kg/min, was administered. When a plateau of secretion had been reached, distention was produced by inflation of the balloon with 60–70 cc of air. The distention of the colon was previously checked by fluoroscopy during the operation and was not enough to cause pain during the experiment. After five collections during the distention of the left colon, the balloon was deflated and five more collections were taken.

Stage II. A Foley catheter was inserted into the right-colonic Thiery loop through the opening in the abdominal wall, and the experiment was done in the same manner as described in stage I. In addition, in a separate group of experiments, the effects of atropine (1 mg, intravenous), α-adrenergic block (phentolamine, 2 mg, intravenous), and β-adrenergic block (propranolol, 2 mg, intravenous) on the inhibitory action of colonic distention was studied. Each drug was tested on a different day.

Each sample of pancreatic fluid collected was analyzed individually for volume, bicarbonate, and protein, the latter serving as a measure of enzyme secretion. Bicarbonate was measured with a Beckman Cl/Co$_2$ analyzer, and protein determination was performed as described by Preshaw and associates (7). Statistical significance was determined by Student's *t* test.

Results

Stage I. Left-colon distention caused no significant changes in the flow or in the protein and bicarbonate concentration and output of secretin-stimulated pancreatic secretion (Table 1).

Stage II. Right-colon distention with submaximal doses of secretin (0.03 unit/kg/min) induced an inhibition of flow of 44% ($p < 0.01$) (Fig. 1; Table 2). This effect was observed immediately after distention of the right colon. Bicarbonate concentration was reduced by 8% ($p < 0.01$) and output by 48% ($p < 0.001$) (Table 3; Fig. 1). Protein concentration decreased 65% ($p < 0.001$), and protein output decreased 82% ($p < 0.001$) (Table 4; Fig. 1). Following deflation of the balloon, all these changes returned to basal values.

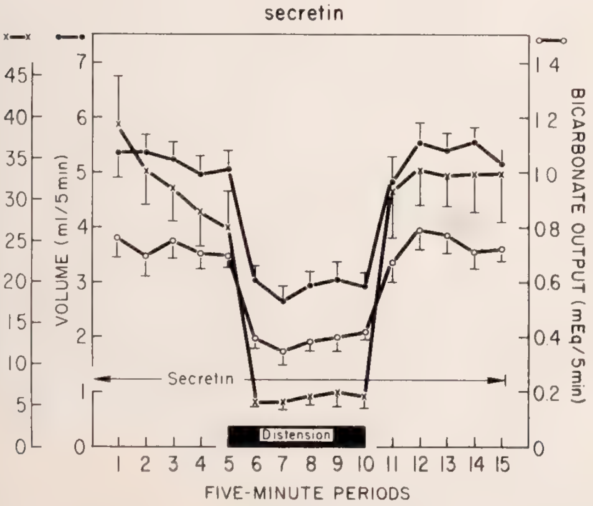


Fig. 1. Left Right colon distention inhibits secretin-stimulated pancreatic flow, bicarbonate concentration, and protein output.

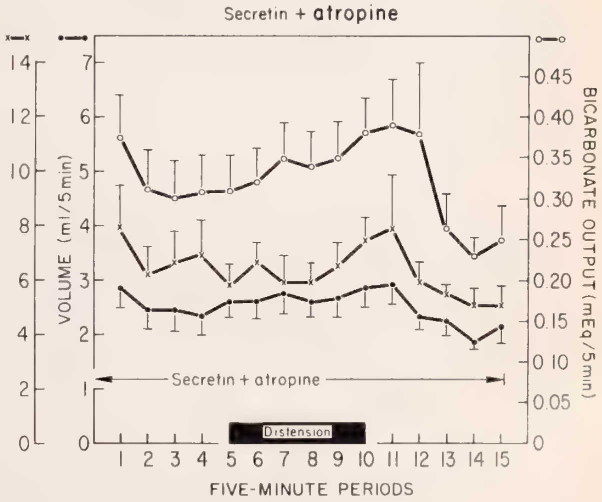


Fig. 2. Right Atropine blocks inhibitory effect of right-colon distention on secretin-stimulated pancreatic secretion.

TABLE 3

Effect of Right-Colon Distention on Pancreatic Bicarbonate Secretion

	No. exp.	Bicarb concentration (mEq/liter)			Bicarb output (mEq/5 min)		
		Control	Distention	Change	Control	Distention	Change
Secretin	11	139 ± 3	130 ± 4	8%*	0.7 ± 0.05	0.4 ± 0.1	−48%*
Secretin + atropine	11	122 ± 4	122 ± 4	+1%	0.3 ± 0.05	0.34 ± 0.04	+6%
Secretin + phentolamine	6	116 ± 8	105 ± 7	−11%*	0.6 ± 0.1	0.3 ± 0.06	−47%*
Secretin + propranolol	9	129 ± 2	114 ± 4	−11%*	0.63 ± 0.07	0.26 ± 0.03	−59%*

* $p < 0.01$.

Atropine, 1 mg intravenous, blocked the inhibitory effect of right-colon distention (Fig. 2). No changes in flow, bicarbonate, and protein concentration were observed. The administration of an α-adrenergic blocker (phentolamine) did not block the inhibitory effect of right-colon distention (Tables 2–4), nor did the administration of a β-adrenergic blocker (propranolol) affect the inhibition of secretin following right-colon distention (Figs. 3, 4; Tables 2–4).

Discussion

The studies demonstrated that mechanical distention of the left colon did not inhibit secretin-stimulated pancreatic exocrine secretion. On the other hand, right-colon distention was followed by a significant decrease in flow and bicarbonate and protein concentration and output. This effect of right-colonic distention was immediate and observed within 5 min. Return to basal secretion after release of the balloon occurred within 10 min. Intravenous atropine blocked the inhibitory effect of right-colon distention on flow and bicarbonate and protein concentration and output; administration of α and β blockers did not.

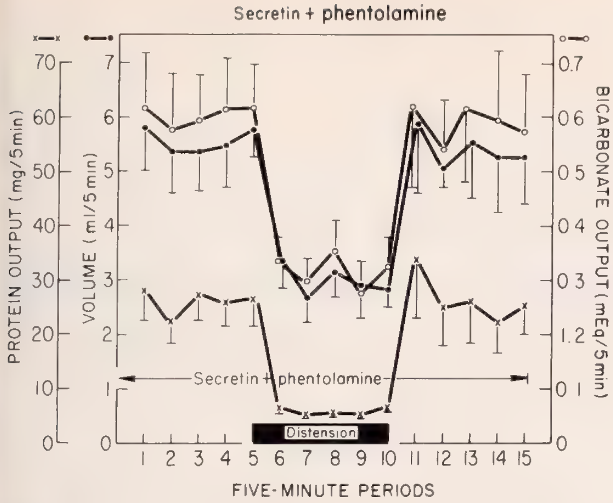
The inhibitory effect on pancreatic exocrine secretion observed by others following infusion of oleic acid into the colon has been attributed to a humorally mediated mechanism, since the inhibition persists after denervation of the pancreas and gut (8–10). The hormone implicated by some is pancreatic polypeptide. Indeed, small amounts of pancreatic polypeptide are found in the intestinal tract from the esophagus to the colon (11, 12). The inhibitory effect of increased serum pancreatic polypeptide on pancreatic exocrine secretion elicited by infusion of liver extract into a Thiry-Vella loop (13–16) can be abolished by intravenous atropine (17). These reports are consistent with the hypothesis of a cholinergic-mediated enteric phase of pancreatic secretion and are in accordance as well with the data presented. The different responses observed in left- and right-colon distention can be explained by the distribution of the vagal innervation; that is, the vagus supplies only the right colon. The failure of distention of the left colon to alter secretin-stimulated pancreatic secretion supports

TABLE 4

Effect of Right-Colon Distention on Pancreatic Protein Secretion

	No. exp.	Protein concentration (mg/ml)			Protein output (mg/5 min)		
		Control	Distention	Change	Control	Distention	Change
Secretin	11	5.8 ± 0.6	2 ± 0.2	−65%*	32 ± 4.5	5.9 ± 0.9	−82%*
Secretin + atropine	11	2.7 ± 0.3	2.4 ± 0.1	−9.7%	6.8 ± 1	6.7 ± 0.8	−2%
Secretin + phentolamine	6	5.2 ± 0.6	2.1 ± 0.3	−59%*	27 ± 6	5.9 ± 0.7	−78%*
Secretin + propranolol	9	5.8 ± 0.5	3 ± 0.3	−46%*	29 ± 4	6.7 ± 0.9	−77%*

* $p < 0.01$.



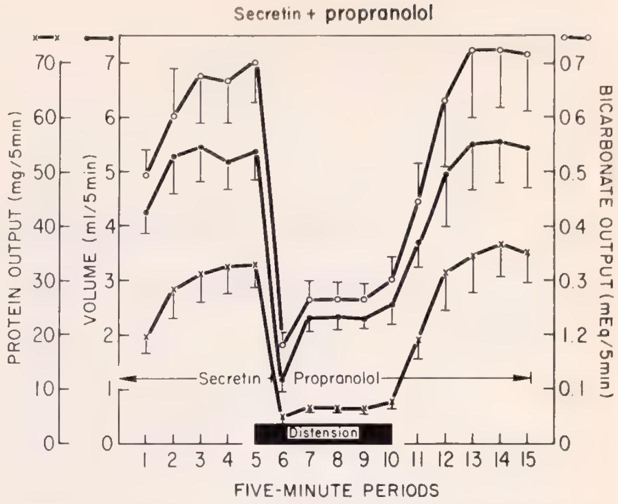


Fig. 3. **Left** Phentolamine does not block inhibitory effect of right-colon distention on secretin-stimulated pancreatic secretion.

Fig. 4. **Right** Propranolol does not block inhibitory effect of right-colon distention on secretin-stimulated pancreatic secretin.

the existence of a coloenteropancreatic reflex (18). The fact that distention of the right colon produces a reflex blocked by atropine indicates cholinergic mediation. However, participation of pancreatic polypeptide or other hormonal mediators cannot be excluded by the data (12).

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Enteropancreatic Reflexes
5: The Effect of Gallbladder and Common Bile Duct Distention on Pancreatic Exocrine Secretion

MOSHE KOLLER, M.D., ANDREW T. TRIEBLING, M.D., LEWIS TOTH, PH.D., AND DAVID A. DREILING, M.D.

Abstract

Increased intraluminal gallbladder and common bile duct pressure caused by balloon distention was studied in anesthetized dogs. A Foley catheter was inserted into the gallbladder and a Fogarty catheter into the common duct. The study was done in the secretin-stimulated state. Pancreatic secretion was collected before and during distention. There was no change in pancreatic secretion due to distention of these structures, in contrast to the effect of distention in other parts of the gastrointestinal tract.

The effect of mechanical distention of almost the entire gastrointestinal tract, i.e. stomach (1), duodenum (2), small bowel (3, 4), and large bowel (5), has been shown to inhibit or stimulate enteropancreatic reflexes by neural and possibly neurohormonal mechanisms. Thus, there is a correlation between release of cholecystokinin-pancreozymin, gallbladder pressure, and pancreatic protein secretion (6). Both stimulatory and inhibitory effects on the gallbladder have been demonstrated in various species for the parasympathetic and sympathetic system, respectively (7). It is, therefore, likely that hormonal and neural influences have physiologic importance in the control of gallbladder motility (8). The purpose of the present study is to evaluate the effect of mechanical distention of gallbladder and common duct on pancreatic exocrine secretion.

Materials and Methods

Acute experiments were carried out on eight healthy mongrel dogs weighing 17–20 kg. They were fasted for 36 hr and were anesthetized with pentobarbital sodium, 30 mg/kg intravenously. The abdomen was opened, and a Foley catheter was inserted into the gallbladder as a cholecystostomy tube. Then the duodenum was opened, the accessory duct was ligated, and a glass cannula was inserted into the main pancreatic duct. Intravenous infusion of 0.45% sodium chloride was maintained throughout each experiment at a rate of 50 cc/hr to allow for fluid losses. The effect of distention of the gallbladder and the common duct was studied under secretin stimulation in doses of 0.03 units/kg/min (Secretin Kabi). Pancreatic secretion was collected at 10-min intervals, and when plateau was reached, distention of the gallbladder was produced by inflation of the Foley catheter balloon with 50 cc of air. Distention of the common duct was achieved by inflation of the Fogarty catheter balloon by 1 cc of air. Distention was verified during the operation. After 5-min collections during distention, the balloon was released and five more 10-min collections were taken.

In these studies, volume was measured as

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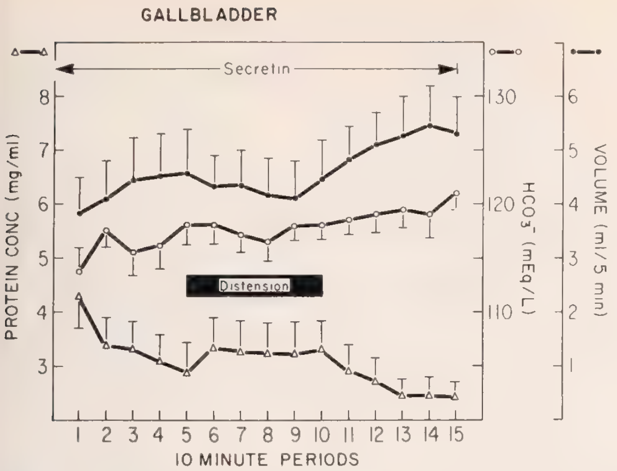

Fig. 1. Distention of gallbladder does not influence secretin-stimulated pancreatic flow or bicarbonate and protein concentration.

ml/10 min, bicarbonate concentration was measured with a Beckman CO_2 analyzer as mEq/liter, and protein concentration was measured as mg/ml. The mean values for volume and bicarbonate and protein concentration of consecutive 10-min collections gathered before distention were compared with those collected during distention. Statistical evaluation was performed using Student's *t* test for unpaired values.

Results

Gallbladder Distention. Distention of the gallbladder produced no significant alteration in secretin-stimulated pancreatic secretion: the change in flow was −0.3% (from 4.28 ml/5 min to 4.27 ml/5 min), in bicarbonate concentration +0.9% (116 mEq/liter to 117 mEq/liter), and in protein concentration −3.6% (3.4 mg/ml to 3.28 mg/liter) (Fig. 1; Table 1).

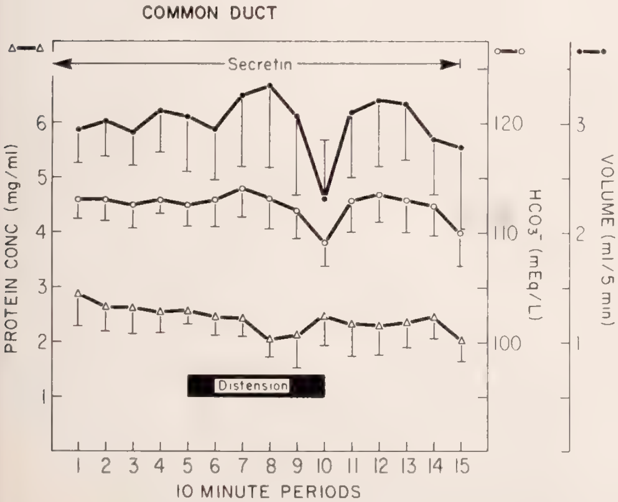

Fig. 2. Distention of common bile duct does not influence secretin-stimulated pancreatic flow or bicarbonate and protein concentration.

TABLE 1

The Effect of Gallbladder Distention on Secretin-Stimulated Pancreatic Secretion
(Secretin 0.03 unit/kg/min)*

		Control	Distention	% Change
Volume	ml/5 min	4.28	4.27	−0.3%*
Bicarbonate	mEq/liter	116	117	+0.96%*
Protein	mg/ml	3.4	3.28	−3.6%*

* No significant statistical difference.

Common Bile Duct Distention. Distention of the common bile duct caused no significant effect on secretin-stimulated pancreatic secretion. The change in volume was −1.1% (3.0 ml/5 min to 2.96 ml/5 min), in bicarbonate concentration −0.9% (113 mEq/liter to 112 mEq/liter), and in protein concentration −11% (2.6 mg/liter to −2.3 mg/ml) (Fig. 2; Table 2).

Discussion

This study indicates that mechanical distention of the gallbladder and the common duct does not affect secretin-stimulated pancreatic secretion. Previous studies have shown that gastric distention causes an increase in pancreatic secretion (1,9). Duodenal distention, on the other hand, has not affected pancreatic secretion (2). Finally, inhibition of pancreatic secretion was observed following distention of the jejunum, ileum, and right colon (3–5). From the data presented it was postulated that the latter phenomena represented enteropancreatic reflexes, either mediated vagally or resulting from release of pancreatic polypeptide or some undetected gut hormone (3–5).

The role of CCK-PZ and the parasympathetic nervous system in the regulation of biliary tract function is well established. In response to intraduodenal fat, significant elevation of plasma cholecystokinin, gallbladder pressure, and pancreatic protein secretion has been reported (9). The human gallbladder empties rapidly after intra-

TABLE 2

The Effect of Common Duct Distention on Secretin-Stimulated Pancreatic Secretion
(Secretin 0.03 unit/kg/min)*

		Control	Distention	Change
Volume	ml/5 min	3	2.96	−1.1%*
Bicarbonate	mEq/liter	113	112	−0.9%*
Protein	mg/ml	2.6	2.3	+11.6%*

* No significant statistical difference.

duodenal fat (10) and, of course, in response to exogenous CCK-33 (11). Moreover, gallbladder size increases after truncal vagotomy but not after selective vagotomy, indicating parasympathetic involvement in maintenance of gallbladder tone (12). Contraction of the gallbladder distends the common duct, reduces choledochal resistance (14), and empties bile into the duodenum, which in turn stimulates pancreatic secretion (13). Distention of the gallbladder empties it of bile and thus mimics gallbladder contraction. It was, thus, surprising that cholecystic and choledochal distention did not affect pancreatic secretion. The mechanics of digestion would suggest that increased pancreatic flow is appropriate, so that detergent and enzyme enter the duodenum simultaneously to initiate the degradation of foodstuffs. On the other hand, inhibition of secretion, as seen in the midgut, would be surprising, since it would reduce pancreatic duct pressure and favor biliary-pancreatic ductal regurgitation.

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Enteropancreatic Reflexes 6: The Effect of Esophageal Distention on Gastric and Pancreatic Secretion

MOSHE KOLLER, M.D., CHENG-HSI SU, M.D., LEWIS TOTH, PH.D., AND DAVID A. DREILING, M.D.

Abstract

We studied the effect of esophageal distention on exocrine pancreatic secretion and gastric juice secretion in the basal state, in the secretin-stimulated state, and under both conditions with the addition of atropine. Under basal conditions, esophageal distention caused increase of pancreatic flow and protein secretion. Basal gastric flow and acid output were even more markedly augmented. The addition of atropine caused only a small change in the pancreatic exocrine secretion, but gastric secretion was totally inhibited. Esophageal distention following secretin stimulation increased pancreatic flow and protein and bicarbonate secretion. The increase in gastric flow and acid output was of greater magnitude. The addition of atropine caused inhibition of the pancreatic response to esophageal distention and totally inhibited gastric secretion. We conclude that esophageal distention causes an increase in pancreatic and gastric secretion by effects mediated by hormonal mechanisms for the pancreas and by neural mechanisms for the stomach.

The existence of enteropancreatic reflexes induced by diverse stimuli and mediated by different pathways is well documented (1–4). Pancreatic exocrine secretion is responsive to neural as well as hormonal stimulation. In humans, sham feeding elicits augmentation of pancreatic enzyme secretion (1). In dogs, a similar response is observed following either sham feeding or electrical stimulation of the esophagus (2). Serum pancreatic polypetide rises markedly following the ingestion of a meal. Modlin et al (4) demonstrated an enteric phase of pancreatic polypeptide release and suggested that this enteropancreatic reflex is mediated by neural and/or hormonal cholinergic-sensitive mechanisms. Enteropancreatic inhibitory as well as stimulatory reflexes have been initiated by mechanical distention of the stomach (5), duodenum (6), small bowel (7, 8), large bowel (9), gallbladder, and common duct (10). The purpose of this study is to investigate the effects of esophageal distention on gastric and pancreatic exocrine secretion in the basal state as well as following exogenous secretin stimulation.

Methods

Five adult mongrel dogs weighing 17–20 kg were prepared with Thomas cannulas placed in the duodenum opposite the major pancreatic duct after preliminary ligation of the accessory duct. A gastric cannula was positioned in the dependent part of the stomach to divert gastric secretion from the duodenum during subsequent experiments as well as to collect aliquots of gastric juice. At the same time, an esophageal fistula was prepared and opened 10 days later. A period of at least three weeks was allowed for the animals' recovery from these procedures before experiments were started. A continuous intravenous infusion of 0.45% sodium chloride was maintained throughout each experiment at a rate of 50 ml/hr to allow for fluid losses.

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TABLE 1

Effect of Esophageal Distention on Basal Pancreatic Secretion

	No. exp.	Distention Before	Distention During	Change
Volume (ml/15 min)	9	0.5 ± 0.2	2.7 ± 0.4	+440%*
Protein (mg/15 min)	9	37.0 ± 10	179 ± 31	+384%*

* $p < 0.001$.

TABLE 2

Effect of Esophageal Distention on Secretin-Stimulated Pancreatic Secretion

	No. exp.	Distention Before	Distention During	Change
Volume (ml/15 min)	6	7.5 ± 1.4	11.5 ± 1.1	+53%*
Protein (mg/15 min)	6	42.0 ± 11.0	160.0 ± 49.4	+280%*
Bicarbonate (mEq/15 min)	6	1.0 ± 0.24	1.5 ± 0.2	+50%*

* $p < 0.05$.

The effect of esophageal distention was studied in the basal state and following secretin stimulation. Pancreatic secretion was collected by direct cannulation through the Thomas cannula at 15-min intervals. Secretin (Kabi vitrum AB), in doses of 0.03 unit/kg/min, was administered. When a plateau of secretion was reached, distention was produced by insertion of a Foley catheter through the esophageal fistula. The Foley balloon was inflated with 50 ml of air, and distention was maintained for 15 min, during which gastric and pancreatic secretion were collected. Following distention, six additional 15-min specimens of gastric and pancreatic secretion were obtained. This protocol was performed in the same canine under basal conditions, following secretin stimulation with and without atropinization (1 mg intravenous bolus followed by continuous intravenous drip of 0.05 mg/kg/hr).

TABLE 3

Effect of Esophageal Distention on Basal Pancreatic Secretion with Atropinization

	No. exp.	Distention Before	Distention During	Change
Volume (ml/15 min)	11	0.2 ± 0.04	0.9 ± 0.2	+384%*
Protein (mg/15 min)	11	3.1 ± 0.6	13.2 ± 3.0	+330%*

* $p < 0.001$.

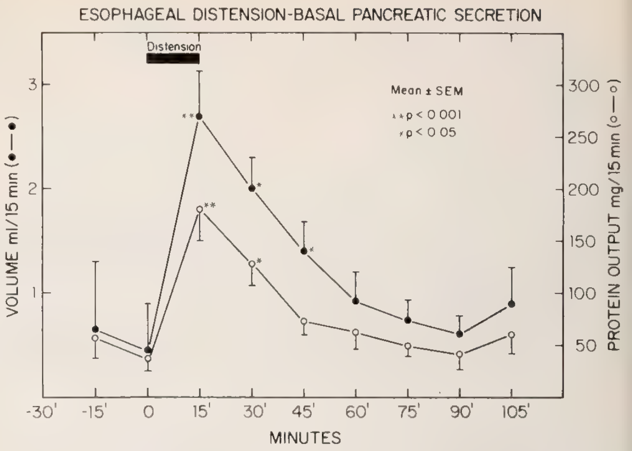

Fig. 1. Esophageal distention; basal pancreatic secretion.

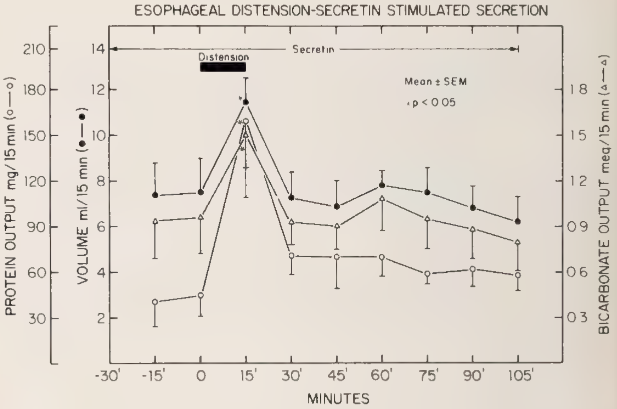

Fig. 2. Esophageal distention; secretin-stimulated secretion.

Each sample of pancreatic fluid collected was analyzed individually for volume and bicarbonate and protein concentrations, the latter serving as a measure of enzyme secretion. Bicarbonate was determined by Technicon autoanalyzer-II, and protein concentration was obtained

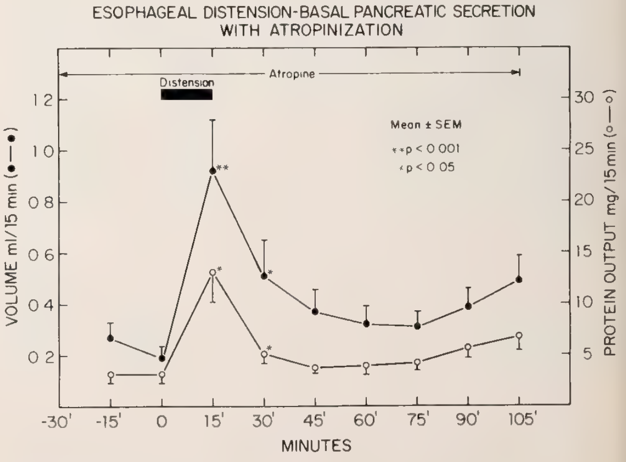

Fig. 3. Esophageal distention; basal pancreatic secretion with atropinization.

TABLE 4
Effect of Esophageal Distention on Secretin-Stimulated Pancreatic Secretion with Atropinization

	No. exp.	Distention		Change
		Before	During	
Volume (ml/15 min)	6	7.1 ± 1.0	4.8 ± 1.4	−32%*
Protein (mg/15 min)	6	14.4 ± 1.2	9.8 ± 1.8	−32%*

* $p < 0.05$.

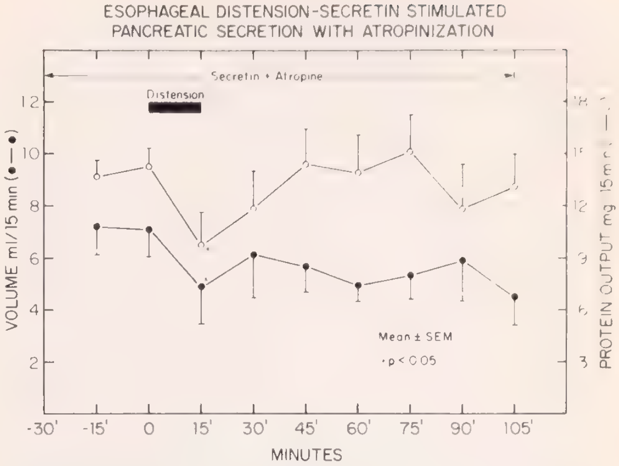

Fig. 4. Esophageal distention; secretin-stimulated pancreatic secretion with atropinization.

by Gilford spectrophotometer at 280-nm wavelength. Gastric specimens were analyzed individually for volume and acid output. Acid concentration was measured with the automatic titrator (Radiometer Copenhagen). Statistical analysis was by standard Student's t test.

Results

Pancreatic Secretion. Esophageal distention under basal conditions resulted in a statistically significant ($p < 0.001$), fivefold increase in pancreatic flow (+440%) and protein output (+384%) (Table 1, Fig. 1). This increase continued after distention was discontinued. The increases were statistically significant, for flow at 45 min and for protein secretion at 30 min. Bicarbonate output could not be reliably determined because of low basal flows.

The effect of esophageal distention on secretin-stimulated pancreatic secretion was a statistically significant ($p < 0.05$) increase in pancreatic flow (+53%) and bicarbonate secretion (+50%) and a close-to-threefold increase in protein secretion (+280%). After distention was discontinued, flow and bicarbonate secretion promptly returned to predistention levels. Although protein output also declined, the rate of secretion remained statistically significantly ($p < 0.05$) elevated above the secretin-stimulated plateau for 3 hr (Table 2, Fig. 2).

Previous atropinization did not significantly alter the response of basal pancreatic secretion to esophageal distention. Both flow (+384%) and rate of protein secretion (+330%) were increased more than threefold. The changes were statistically significant ($p < 0.001$), but the increases were less prolonged and significant for only 15 min (Table 3, Fig. 3). After release of distention, flow and protein output remained slightly elevated.

Atropinization prior to secretin stimulation reversed the effects of esophageal distention. Instead of augmentation, the result was a significant ($p < 0.05$) inhibition of flow (−32%) and protein output (−32%) responses to secretin. The secretin effect, however, was not abolished and continued well above basal levels. After release of distention, the inhibition lasted for 15 min at a rate that was not statistically significant (Table 4, Fig. 4).

Gastric Secretion. Esophageal distention resulted in an eightfold increase in gastric flow (+700%) and a 17-fold increase in acid output

TABLE 5
Effect of Esophageal Distention on Basal Gastric Secretion

	No. exp.	Distention		Change
		Before	During	
Volume (ml/15 min)	9	2.2 ± 0.6	17.6 ± 2.7	+700%*
Acid output (mEq/15 min)	8	0.1 ± 0.05	1.2 ± 0.3	+1,600%*

* $p < 0.01$.

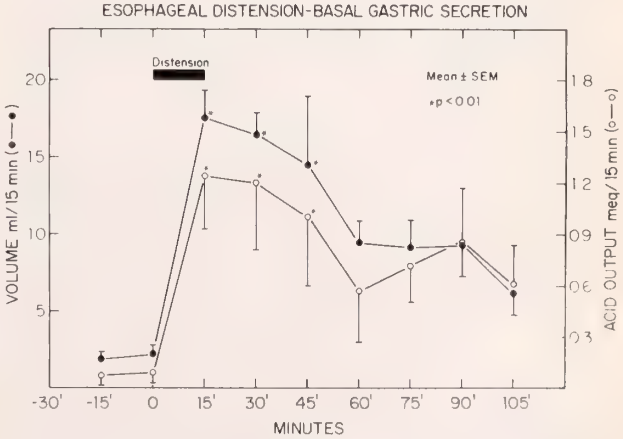

Fig. 5. Esophageal distention; basal gastric secretion.

TABLE 6
Effect of Esophageal Distention on Secretin-Stimulated Gastric Secretion

	No. exp.	Distention Before	Distention During	Change
Volume (ml/15 min)	6	1.0 ± 0.38	9.8 ± 1.7	+900%*
Acid output (mEq/15 min)	5	0.2 ± 0.06	0.73 ± 0.26	+2,650%*

* $p < 0.01$.

(+1,600%). This augmentation was statistically significant ($p < 0.01$) for 45 min (Table 5, Fig. 5).

Esophogeal distention following the administration of secretin resulted in a 10-fold increase in gastric flow (+900%) and a 35-fold increase in acid output (+2,650%). These increases were statistically significant ($p < 0.01$) for 90 min after release of distention (Table 6, Fig. 6).

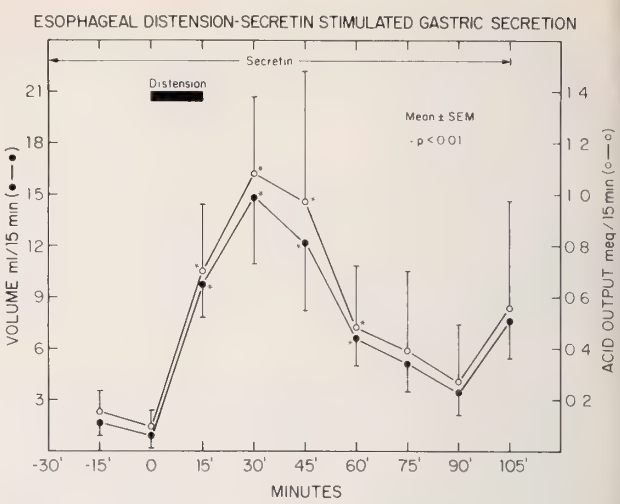

Fig. 6. Esophageal distention; secretin-stimulated gastric secretion.

Previous atropinization totally inhibited the effects of esophageal distention on basal gastric secretion as well as secretin-stimulated gastric secretion.

Discussion

Control of pancreatic exocrine secretion has been exhaustively studied in the past decades (11). In contrast to control of gastric secretion, which appears to be primarily neural, the pancreatic exocrine secretion is controlled by a combination of neural and hormonal actions. The control mechanisms are usual sequentially described as cephalic, gastric, and intestinal phases. The cephalic phase comprises reflexes produced by olfactory and visual stimuli mediated by efferent fibers in the vagus nerve (12). The gastric phase is by neural (vagal) and hormonal (gastrin) stimulation (13–15). In the intestinal phase, there are both stimulatory and inhibitory effects. Duodenal acidity increases pancreatic bicarbonate secretion and the plasma level of secretin (16–18). In humans also, intraduodenal infusion of bile salts stimulates pancreatic enzymes and plasma secretin (19–21). Inhibitory effects associated with a rise in serum pancreatic polypeptide levels are achieved by an infusion of oleic acid into the small and large bowel (22–25) and by mechanical distention of the distal part of the duodenum (16), jejunum (17), ileum (18), and right but not left colon (9).

The role of the esophagus and its influence on pancreatic secretion have not been previously investigated. In the present study, mechanical distention of the esophagus caused increased pancreatic exocrine secretion, in both the basal and the secretin-stimulated state. This stimulation lasted for a longer period in the unstimulated secretion, probably because of the masking effect of the secretin. Atropine did not abolish the response of pancreatic secretion, and therefore it seems likely that this effect is hormonally mediated.

On the other hand, esophageal distention caused increases in gastric secretion and acid output, an effect that was totally abolished by atropinization. Thus, esophageal distention appears to influence gastric and pancreatic secretion in different ways, gastric secretion by neural and pancreatic by hormonal mechanisms.

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Enteropancreatic Reflexes
7: The Effect of Duodenal Distention on Pancreatic Exocrine Secretion

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Abstract

The effect of duodenal distention on pancreatic exocrine secretion was studied in a group of dogs with chronic pancreatic and gastric fistulas. In the secretin-stimulated experiments, distention of the distal duodenum reduced pancreatic flow 44%, protein output 43%, and bicarbonate output 44%. Distention of the proximal duodenum, however, induced no significant change. The inhibitory effect of distal duodenal distention may be mediated, at least in part, by an atropine-sensitive cholinergic reflex.

It has been shown that duodenal infusion of hypertonic glucose solution depresses the secretin-stimulated pancreatic secretion in dogs (1), whereas intraduodenal perfusion of long-chain fatty acids produces increases of pancreatic protein output as well as of pancreatic bicarbonate secretion in humans (2) and in the dog (3). Oleic acid perfusion in the duodenum, however, results in decreased protein output at the end of perfusion (4). Sum et al (5) report that balloon distention of the duodenum increases gastric secretion but has no effect on pancreatic exocrine secretion. On the other hand, enhanced pancreatic secretion in response to gastric distention has been demonstrated in dogs (6–8) and humans (9). Recently, Lambert and Hansky (10) observed that proximal small-intestinal distention induced pancreatic secretion. The same result was found in jejunal (11), ileal (12), and right-colon (13) distention in our laboratory. The aim of the present study is to reevaluate the effect of duodenal distention on pancreatic exocrine secretion.

Materials and Methods

Four adult mongrel dogs weighing 14–20 kg were prepared with a Thomas cannula in the duodenum sited opposite the pancreatic duct after preliminary ligation of the accessory duct. At the same time, a gastric cannula was positioned in the most dependent part of the stomach to divert gastric secretion from the duodenum during subsequent experiments.

The experiments were started more than three weeks after surgery. The dogs were fasted for 18 h and then stood in sling harnesses during each test. A continuous intravenous infusion of 0.9% of sodium chloride was maintained throughout each experiment. The pancreatic duct was selectively cannulated, and pure pancreatic juice was collected. The gastric cannula was opened. Secretin (KABI), in a dose of 1 unit/kg/hr, was infused continuously. When a plateau was reached, a Foley catheter was inserted into the duodenum through the Thomas cannula. Distal duodenal distention was produced by inflation of the balloon with 50 cc of air about 10 cm distal to the pancreatic papilla. It was distended for 15 min and then deflated. The pancreatic juice

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was collected in 15-min intervals for 90 min after distention. Proximal duodenal distention was produced by the same method with the Foley catheter inserted 5 cm proximal to the papilla. In separate experiments, duodenal distention was tested with intravenous atropinization superimposed on a secretin-stimulated background. Atropine was given in a 50 μg/kg bolus followed by a 20 μg/kg/hr continuous infusion.

Each collected pancreatic sample was analyzed individually for volume, protein, and bicarbonate concentration. The protein concentration was taken as a measure of enzyme secretion. Bicarbonate was measured with Technicon autoanalyzer II, and protein determination was performed by Gilford Spectrophotometer at 280-nm wavelength.

Statistical significance was studied by paired Student's t test between control values (15 min before distention) and those obtained during and following duodenal distention with or without atropine. In all the tests, data were expressed as mean ± standard error of the mean.

Results

Distal Duodenal Distention. Duodenal distention resulted in 44% reduction of volume, 43% reduction of total protein output, and 44% reduction of bicarbonate output of secretin-stimulated exocrine secretion. The inhibitory effect was statistically significant for 75 min after deflation of the balloon (Table 1, Fig. 1). This intubation was totally abolished by atropinization during and after duodenal distention (Table 2, Fig. 2).

Proximal Duodenal Distention. Owing to technical difficulty, the pancreatic juice could not be collected during proximal duodenal distention. Only the postdistention collections were studied.

Secretin-stimulated exocrine secretion following proximal duodenal distention resulted in a 19% decrease in volume, 15% decrease in bicarbonate output, and no change in the protein output, alterations not statistically significant (Table 3, Fig. 3). Following atropinization, the pancreatic juice and bicarbonate output decreased 6% and 9%, respectively. The protein output increased 15%. Again, these changes were not statistically significant (Table 4, Fig. 4).

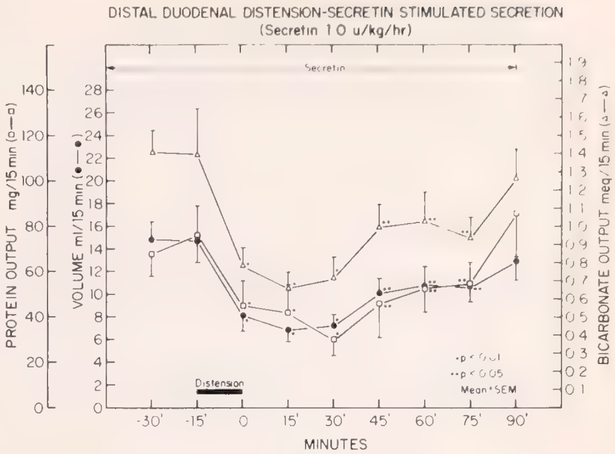

Fig. 1. Effect of distal duodenal distention on pancreatic volume, protein and bicarbonate output of secretin-stimulated secretion. Mean ± SEM of 9 experiments. Asterisk denotes significant difference from basal (*$p < 0.01$, **$p < 0.05$).

Discussion

It has been shown that distention of the proximal stomach initiates a gastropancreatic reflex and results in an increase in pancreatic secretion (6, 7, 9). The same effect is also found in antral distention, which is believed to occur via cholinergic-mediated pyloropancreatic reflexes (8, 14–17).

The studies of Lambert and Hansky (10) and our previous studies (11–13) of sequential distentions of the gastrointestinal tract revealed that distention of jejunum, ileum, and right colon result in an inhibition of pancreatic secretion, whereas distention of the left colon (13), gallbladder, and common bile duct (18) result in no change in secretion. The decreased secretion we

TABLE 1

Effect of Distal Duodenal Distention on Secretin-Stimulated Pancreatic Secretion (Secretin 1.0 unit/kg/hr)

	No. exp.	Distention (mean ± SE)				
		Before	During	Change	After*	Change
Volume (ml/15 min)	9	14.7 ± 1.88	8.3 ± 1.42	−44%†	8.1 ± 0.69	−45%†
Protein (mg/15 min)	9	78.8 ± 13.21	44.6 ± 10.87	−43%†	39.3 ± 6.80	−50%†
Bicarbonate (mEq/15 min)	9	1.4 ± 0.25	0.8 ± 0.11	−44%†	0.8 ± 0.07	−43%†

* Mean value of 15, 30, 45 min after distention.
† $p < 0.01$.

TABLE 2

Effect of Distal Duodenal Distention on Secretin-Stimulated Pancreatic Secretion during and following Atropinization

	No. exp.	Distention (mean ± SEM)				
		Before	During	Change	After*	Change
Volume (ml/15 min)	6	7.8 ± 0.85	8.0 ± 1.31	+3%†	6.5 ± 0.35	−15%†
Protein (mg/15 min)	6	17.1 ± 1.72	21.9 ± 3.12	+27%†	15.7 ± 1.11	−8%†
Bicarbonate (mEq/15 min)	6	0.9 ± 0.17	1.0 ± 0.21	+14%†	0.8 ± 0.06	−16%†

Secretin 1.0 unit/kg/hr; atropine 50 µg/Kg bolus; infusion at 20 µg/kg/hr.

* Mean value of 15, 30, 45 min after distention.

† $p > 0.01$.

TABLE 3

Effect of Proximal Duodenal Distention on Secretin-Stimulated Pancreatic Secretion (Secretin 1.0 unit/kg/hr)

	No. exp.	Distention (mean ± SEM)		
		Before	After*	Change
Volume (ml/15 min)	10	11.6 ± 1.26	9.3 ± 0.88	−19%†
Protein (mg/15 min)	10	44.9 ± 10.56	45.0 ± 8.05	+0.0%†
Bicarbonate (mEq/15 min)	10	1.2 ± 0.10	1.0 ± 0.11	−15%†

* Mean value, 15, 30, 45 min after distention.

† $p > 0.01$.

observed following gut distention was inhibited by atropine (11–13, 18), presumably involving atropine-sensitive enteropancreatic reflexes. Recently, distention of the esophagus was shown to augment pancreatic secretion. This was not abolished by atropine; therefore, a humorally mediated factor is postulated (19).

Intraduodenal infusion of a hypertonic glucose solution was reported to depress secretin-stimulated pancreatic secretion in anesthetized dogs (1). Laugier and Sarles (4) demonstrated that intraduodenal infusion of oleic acid induced an increased pancreatic flow and bicarbonate secretion, the protein output initially increasing mildly, then significantly decreasing. Sum et al (5) reported that no effect on external pancreatic secretion followed distention of the duodenum by balloon. In our studies, however, mechanical distention of the proximal duodenum did affect secretin-stimulated pancreatic secretion. On the

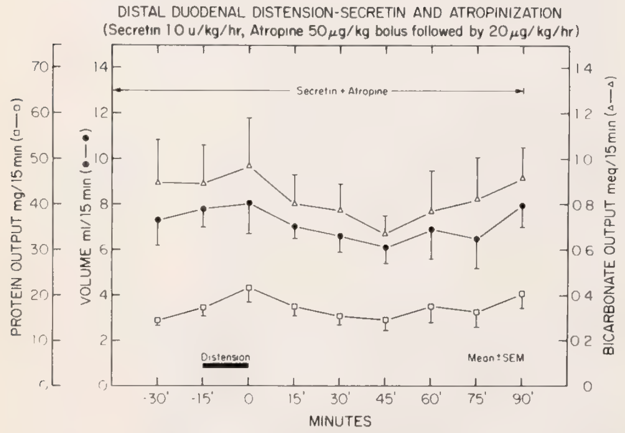

Fig. 2. Effect of distal duodenal distension on pancreatic volume, protein and bicarbonate output of secretin-stimulated secretion with atropinization. Mean ± SEM of 6 experiments.

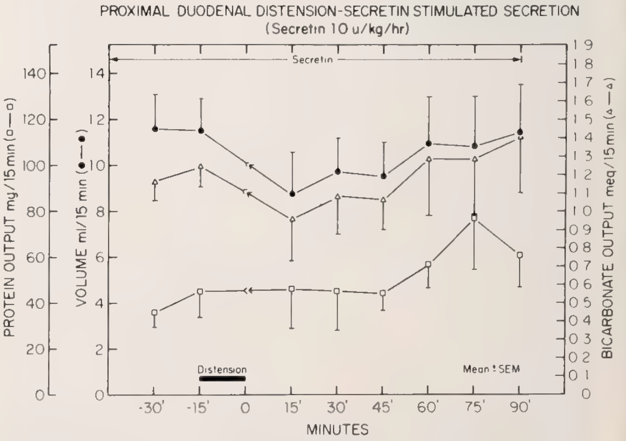

Fig. 3. Effect of proximal duodenal distension on pancreatic volume, protein and bicarbonate output of secretin-stimulated secretion. Mean ± SEM of 10 experiments.

TABLE 4

Effect of Proximal Duodenal Distention on Secretin-Stimulated Pancreatic Secretion during and following Atropinization

	No. exp.	Distention (mean ± SEM)		
		Before	After	Change
Volume (ml/15 min)	6	8.6 ± 1.36	8.0 ± 0.59	−7%†
Protein (mg/15 min)	6	9.3 ± 1.09	10.8 ± 0.85	+16%†
Bicarbonate (mEq/15 min)	6	1.2 ± 0.21	1.1 ± 0.09	−9%†

Secretin 1.0 unit/kg/hr; atropine 50 μg/kg bolus; infusion at 20 μg/kg/hr.

* Mean value of 15, 30, 45 min after distention.

† $p > 0.01$.

other hand, distal duodenal distention significantly decreased pancreatic flow and protein and bicarbonate output, effects that were blocked by atropine. It is possible that Sum et al (5) missed the induction of secretin-stimulated pancreatic juice following duodenal distention by failing to collect pancreatic juice during the first 60 min of distention.

Harper et al (20) showed that stimulation of afferent vagal fibers from the lower end of the gut reflexly inhibited pancreatic secretion. Belching, vomiting, and restlessness were noticed in the cats during duodenal distention. Harper et al (20) suggested that increased sympathetic stimuli may play a role in the inhibitory effect. However, in our previous studies (11–13) the inhibitory effect of jejunal, ileal, and right-colonic distention on pancreatic secretion was not abolished by α- and β-adrenergic blocking agents. The effect of distention was blocked by atropine only. Indeed, tension receptors in the muscle wall of the jejunum initiating afferent impulses in the vagus have been demonstrated in cats (21). Previous studies, moreover, also show the existence of vagally mediated gastropancreatic and pyloropancreatic reflexes (6, 8). Finally, Solomon and Grossman (22) demonstrated that long vagovagal reflexes are responsible for the majority of the exocrine pancreatic response to intraluminal stimuli in the gut by amino acid and lipid. In our study, the inhibitory effect of distal duodenal distention is also blocked by atropine. The data suggest that it must, in part, be cholinergically mediated.

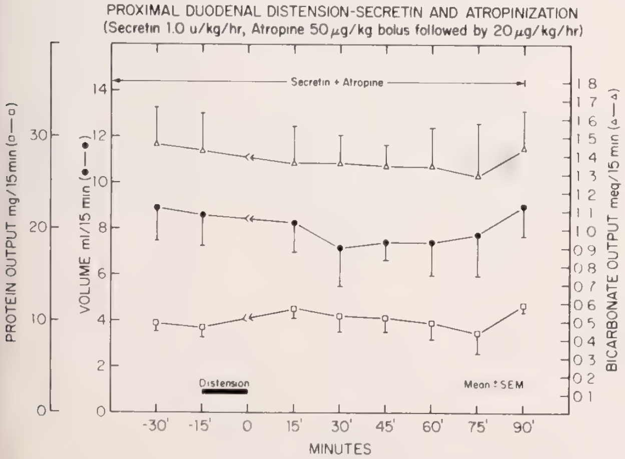

Fig. 4. Effect of proximal duodenal distension on pancreatic volume, protein and bicarbonate output of secretin-stimulated secretion with atropinization. Mean ± SEM of 6 experiments.

The mechanisms for the different effects of distal and proximal duodenal distention are obscure. Our previous observations have shown no inhibition of pancreatic secretion following gallbladder and common bile duct distention (18). Because of anatomic proximity of the duodenum and extrahepatic biliary structures, it is likely that innervation may be related. Further study of the autonomic nerve innervation of the proximal and distal duodenum may clarify this difference.

The significance of an inhibitory component of the hormonal influence on the exocrine pancreas has been studied recently. Inhibitory effects of the oleic acid infusion in the colon and terminal ileum have been observed in dogs (23), rats (4), cats (20), and humans (24). The effect can also be elicited after section of the vagal and splanchnic nerves in the cat (20) and also demonstrated in cross-circulation experiments in rats (4). It has been postulated that pancreatone, an inhibitor of pancreatic secretion extractable from ileal and colonic mucosa, may be the responsible humoral factor (20). In addition, perfusion of a Thiry-Vella loop with liver extract significantly increases plasma pancreatic polypeptide (PP) (25), small amounts of which have been found throughout the gastrointestinal tract (26) and which inhibits pancreatic secretion (27–30). Yet decreased cholecystokinin release—rather than increased pancreatic polypeptide—has been observed when the colon is perfused with oleic acid (31). Recently, enteroglucagon, vasoactive intestinal peptide, neurotensin, somatostatin, and other factors have been suggested as possible candidates for the humoral effect (32). The participation of a hormonal mediator in duodenal distention was not studied in this experiment. It needs future investigation.

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Exocrine Pancreatic Secretion in Dogs: The Effect of Pirenzepine

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Cholinergic innervation has previously been shown to play an important role in the regulation of exocrine pancreatic secretion. Pirenzepine, an anticholinergic drug, inhibits gastric secretion (1–4), accelerates the healing of peptic ulcers (5), and elicits few typical anticholinergic side effects (3, 6). Data concerning its influence on exocrine pancreatic secretion are scarce and controversial (7). The present study was designed to investigate the effect of pirenzepine on exocrine pancreatic secretion in dogs.

Methods. Three mongrel dogs, weighing 18–20 kg, were prepared with a Thomas cannula gastric fistula, which was kept open throughout the experiments to prevent acid from entering the intestine and releasing endogenous secretin. Another Thomas cannula was placed in the duodenum opposite the papilla of Vater. The secretory studies were started about two months after surgery. The dogs were deprived of food but had free access to water for 24 hours before each study. Pancreatic secretion was collected by direct cannulation of the main pancreatic duct through the Thomas cannula at 20-minute intervals in basal conditions and 10-minute intervals in the experiments using hormonal stimulation.

Pancreatic secretion in the stimulated tests was allowed to reach a steady state during the initial 60 minutes of hormone infusion before the actual experiment was started. Secretin (Boots Co. Ltd.) and cholecystokinin–pancreozymin (GIH, Karolinska Institute) were given at the rate of 1 unit/kg/hr each in constant intravenous infusion, together with 0.9% sodium chloride, 50 ml/hr. Pirenzepine (Boehringer Ingelheim Corp., Ridgefield, Conn.) was injected intravenously as a bolus in the dose of 1.3 or 2.6 mg/kg and followed by an infusion of either 0.24 or 0.48 mg/kg/hr. All tests were performed in duplicate on each of the animals. Volume was measured as ml/20 min in basal secretion studies. Volume and bicarbonate and protein concentrations were examined in the tests that included hormonal stimulation tests. In these studies, volume was measured as ml/10 min, bicarbonate concentrations were determined with a Beckman CO_2 analyzer as mEq/L, and protein concentration was measured as mg/ml. The mean values for volume and concentrations of bicarbonate and protein in consecutive 10-minute collections gathered before administration of pirenzepine were compared with mean values of collections made while the drug was being infused. Statistical evaluation was performed using Student's t test for unpaired values.

Results. Under basal conditions, pancreatic flow decreased following administration of pirenzepine (Fig. 1), but the changes observed were not statistically significant. In experiments with hormonally stimulated secretion, no change in flow was observed following pirenzepine infusion (Fig. 2). Similarly, pirenzepine did not change the bicarbonate concentration. However, pirenzepine did inhibit the secretion of enzyme; the average decrease in protein concentration was about 25% of the preinfusion level. At the lower dose of pirenzepine, the change was statistically significant ($p < 0.001$).

Discussion. The data presented indicate that, in dogs, pirenzepine affects both basal and hormon-

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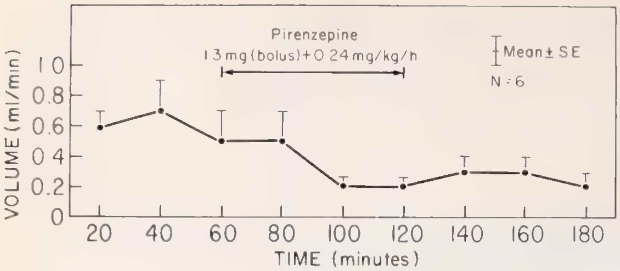

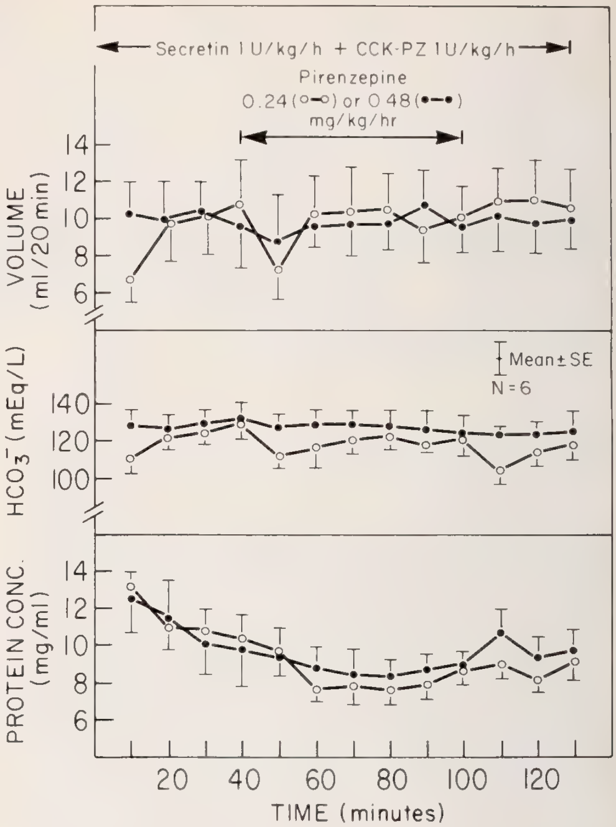

Fig. 1. **Left** Effect of pirenzepine (1.3 and 2.4 mg/kg/hr) on basal pancreatic secretin.

Fig. 2. **Right** Effect of pirenzepine (0.24 or 0.48 mg/kg/hr) on pancreatic flow, bicarbonate concentration, and protein secretion induced by constant infusion of secretin (1 unit/kg/hr) and cholecystokinin-pancreozymin (1 unit/kg/hr).

ally stimulated exocrine pancreatic secretion. In the dosage employed, pirenzepine did not alter hormonally stimulated pancreatic flow or bicarbonate concentration, but did decrease enzyme concentration significantly.

Brunner et al (7) found that in humans, intraduodenally administered pirenzepine induced a short-term increase in the volume of duodenal secretion. The authors postulated a secretin-like action of this substance, suggesting this was possibly a result of a topical effect on the duodenal mucosa. This suggestion was questioned in the light of data reported by W. Londong during a discussion at the Second International Symposium on Pirenzepine (8). In his dose-response studies on peptone stimulation of gastric acid secretion, intravenous pirenzepine did not significantly change the level of endogenous plasma secretin. The different mode of administration could account for the disparity in results. At the same discussion, A. Gabryelewicz reported on his investigations with five patients in whom 0.3 mg/kg of pirenzepine intravenously per hour did not cause significant changes in exocrine pancreatic secretion as evaluated by the standard secretin test.

Atropine, a classical anticholinergic, inhibits secretin-stimulated pancreatic flow rate, decreases bicarbonate, and inhibits protein output (9). Pirenzepine, although a representative of the same group of drugs, seems to possess different properties. In the present study, it selectively decreased protein concentration. This difference may arise from the recent observation that there are subclasses of muscarinic receptors (10), and that atropine binds at sites of both types of receptors, whereas pirenzepine, known to be selective in other organs (11), does not. The experimental data suggest the presence of these muscarinic subtypes in the pancreas.

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Exocrine Pancreatic Secretion in Dogs: Effects of the Synthetic Methionine-Enkephalin Analog FK 33-824 and Opioid-Receptor Antagonist Naloxone

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The greatest concentration of enkephalin found outside the central nervous system is in the gastrointestinal tract (1). This group of endogenous neuropeptides plays some still not fully understood role in gastrointestinal reactions. The natural enkephalins are rapidly and completely degraded by enzymes in various tissue homogenates and in blood; however, some synthetic derivatives are more stable (2, 3).

In animals, the outstanding characteristic of the synthetic methionine(met)-enkephalin analog FK 33-824 is its long-lasting, potent, analgesic effect even after oral administration (4). It inhibits gastrointestinal transit time in rats (5). In humans it exerts powerful dose-related effects on esophageal motility (6). When administered to rats in a dose of 1.5 μg/kg/hr, FK 33-824 stimulated gastric secretion; however, in doses near LD50, pronounced inhibition has been noted (7).

The aim of the present study is to investigate the effect of this new met-enkephalin analog on secretin-stimulated pancreatic exocrine secretion in dogs.

Methods. Three mongrel dogs weighing 18 to 20 kg were each prepared with a gastric fistula drained by a Thomas cannula which was kept open throughout the experiments to prevent acid from entering the intestine and releasing endogenous secretin. Another Thomas cannula was placed in the duodenum opposite the papilla of Vater. Secretory studies were started about two months after surgery. Dogs were deprived of food but had free access to water for 24 hours before each test. Pancreatic secretion was collected by direct cannulation of the main pancreatic duct in 10-minute intervals. Secretin (Boots Co. Ltd.) was given at the rate of 1 unit/kg/hr in constant intravenous infusion, together with 0.9% sodium chloride 50 ml/hr. Pancreatic secretion was allowed to reach a steady state during the initial 60 minutes of hormone infusion. On the background of constant stimulation with secretin, synthetic met-enkephalin analog FK 33-824 (Sandoz, Basel, Switzerland) was intravenously infused in a dose of 1.0 μg/kg/hr alone or with the opioid-receptor antagonist naloxone hydrochloride (Du Pont Pharmaceuticals) in a dose of 40 μg/kg/hr. In a third group of experiments, naloxone alone was infused at the same dose during the midhour of secretin stimulation.

All tests were performed in duplicate on each of the animals. Pancreatic flow and bicarbonate and protein concentration were examined. Bicarbonate was determined with a Beckman CO_2 analyzer. Protein concentration was expressed in mg/ml. The mean values of volume and bicarbonate and protein concentration for 4 consecutive 10-minute collections gathered before administration of the drugs were compared with those collected while the test substance were being infused. Statistical evaluation was by Student's *t* test for unpaired values.

Results. Intravenous infusion of FK 33-824 re-

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sulted in significant decrease ($p < 0.001$) in pancreatic flow: average, 58% (Fig). Mean values of bicarbonate and protein concentration declined by 21% and 57%, respectively. When naloxone, an opioid-receptor anatagonist, was infused alone, the inhibition of pancreatic secretion, although less than after enkephalin, was nevertheless significant ($p < 0.001$). The average decreases were 43% for volume, 12% for bicarbonate concentration, and 22% for protein concentration. In the set of experiments in which the two substances were administered simultaneously, a decrease of 60% in volume secreted and of 21% in bicarbonate concentration was noted. Protein concentration, however, remained unchanged as compared to pretreatment values. About 30 minutes after cessation of the drug infusions, pancreatic secretion returned to pretreatment values.

Discussion. The data presented indicate that the synthetic met-enkephalin analog FK 33-824 given intravenously in a dose of 1 μg/kg/hr causes significant inhibition of secretin-stimulated pancreatic exocrine secretion in dogs. The opioid-receptor antagonist naloxone blocks this inhibitory effect, but only selectively with regard to enzyme secretion. When given alone, naloxone results in inhibition of secretin-stimulated pancreatic secretion.

The inhibitory effect of enkephalin on pancreatic secretion has been reported previously in dogs (8) and in humans (9). In both of these studies, Konturek et al pointed out that this effect elicited by enkephalin was more pronounced whenever the pancreas was stimulated endogenously. The authors suggested that this inhibition might be attributed to the enkephalin-induced suppression of release of endogenous pancreas-stimulating hormones. They have confirmed these phenomena in later studies (10).

The decline in secretin-stimulated pancreatic exocrine secretion observed in these studies was slightly greater than in similar experiments of others (8). The difference may be in the type of enkephalin used. FK 33-824, employed in the present investigations, was shown to be substantially more potent than met-enkephalin in its analgesic effect (4).

In the studies by Konturek et al (9), Ala-2-enkephalin added to intravenous infusions of secretin did not alter protein output in humans. In these studies, a 60% drop in enzyme concentration was noted after enkephalin was administered. The opioid-receptor antagonist naloxone did not prevent the decrease in secretin-stimulated pancreatic flow and bicarbonate concentration evoked by FK 33-824 in these experiments, nor did Ala-2-enkephalin used in other studies (9). Naloxone did, however, block the inhibitory effect of FK 33-824 on protein secretion. Naloxone's higher affinity with "mu" receptors than with the "delta" type could be responsible for this diversity of effects.

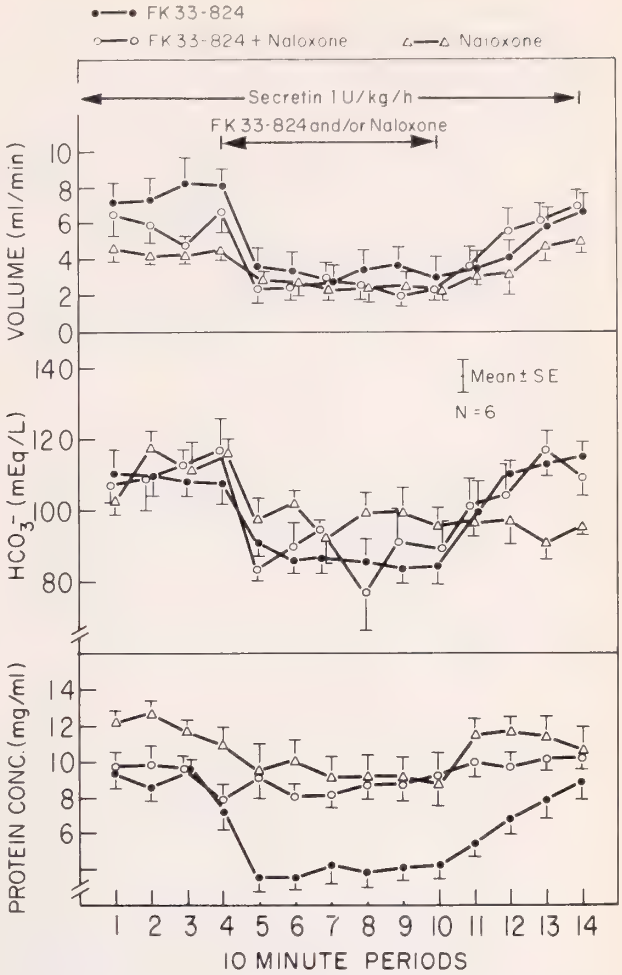

Fig. Effects of enkephalin (FK 33-824), enkephalin plus naloxone, and naloxone alone on pancreatic flow and bicarbonate and protein secretion induced by continuous intravenous infusion of secretin (1 unit/kg/hr).

A surprising observation was that naloxone given alone on the background infusion of secretin caused significant inhibition of pancreatic secretion in the dogs. In humans it did not affect the pancreatic response to secretin (9). Furthermore, in dogs, naloxone intravenous infusion did not significantly alter bicarbonate and protein response to duodenal acid (8). This disparity of results is not understood and requires further clarification.

The mechanism of an opiate's inhibitory effect on pancreatic exocrine secretion is not clear and is likely to be complex. Rapid degradation of endogenous enkephalin by blood proteolytic systems would suggest a local action. Exogenous enkephalin may simultaneously influence pancreatic secretion in at least three different ways. First, it may interfere with cholinergic stimulation of the pancreas, since enkephalin is able to suppress the release of acetylcholine (11). Second, it may block release of endogenous secretin, inasmuch as enkephalin was found to inhibit the rise of blood secretin levels in response to duodenal acidification (10). Third, direct activation of a specific opioid receptor(s) on the pancreatic acinar cells remains a possibility.

Summary. The enkephalin analog studied in canines inhibits secretin-stimulated exocrine pancreatic secretion for flow and bicarbonate and protein concentrations. Naloxone is an antagonist of this effect for protein secretion only. An unexpected observation was the demonstration that naloxone, per se, inhibited pancreatic flow and bicarbonate and protein concentrations.

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Trophic and Antitrophic Circuits Controlling Pancreatic Weight in the Rat

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Abstract

Various surgical procedures were performed on the autonomic nervous system and the digestive tract of male Wistar rats in order to define reflex pathways affecting pancreatic weight. Changes in pancreatic wet weight were compared to weights in six control series of rodents of different body weights. Short-term (3-month) truncal vagotomy elicited a slight increase (+17%) in the weight of the pancreatic head and a marked augmentation (+93%) of the pancreatic body-tail segment. These alterations disappeared in animals with long-term (16-month) vagotomy. Both bilateral splanchnicectomy and celiac ganglionectomy abolished the pancreatic weight increases evoked by short-term truncal vagotomy. The former, by itself, did not elicit pancreatic weight changes. Celiac ganglionectomy induced a weight increase (+46%) of the pancreatic head, but only after a long period (20 months). Long-term alcohol feeding did not modify pancreatic weight, but completely prevented vagotomy-induced pancreatic enlargement. This ethanol-elicited antitrophic effect, presumably deriving from activation of a negative trophic center in the central nervous system and discharging via the spinal cord, the splanchnic nerves, and/or the celiac ganglia, reduced pancreatic weights to levels even below those of controls. Section and reanastomosis of the duodenal wall above and below the bile duct entry into the duodenum (duodenotomy) induced a significant augmentation (+61%) of the pancreatic head weight. This change was less marked when performed at the pylorus (+28%), and without effect when carried out at the fundic-antral junction. Duodenotomy plus resection of the duodenal autonomic nervous brain provoked a simultaneous enlargement of the pancreatic head (+74%) and a reduction of the body-tail segment (−22%). When resection of the antral autonomic nervous brain was performed in addition to duodenal autonomic nervous brain exeresis, the latter pancreatic weight changes were abolished. Pilocarpine (1.0 mg/kg) administered for 10 days to female rats significantly increased (+37%) the body-tail segment. We postulated that functional linking of the antral and duodenal nervous brains, the celiac ganglia, and the pancreatic gland constitutes a small trophic circuit. Its disruption would provoke what we term an "escape" of trophic impulses from the antral autonomic nervous brain to the pancreatic head. On the other hand, the pancreas, the vagal nerves (afferent limb), the central nervous system, the splanchnic nerves, and the celiac ganglia (efferent limb) form a great trophic circuit. Interference with its efferent limb by short-term (3-month) truncal vagotomy triggers the discharge of trophic impulses (adrenergic?) from a positive trophic center in the central nervous system—impulses mainly directed to the pancreatic body-tail segment. Thus, pancreatic weight increases evoked by the partial resection of the gland, chronic trypsin inhibitors or cholestyramine feeding, and chronic diversion of bile-pancreatic juice or bile alone result in a short-term truncal-vagotomy-induced imbalance in the great trophic circuit, with a consequent discharge of trophic impulses from the central nervous system via the spinal cord, the splanchnic nerves, and the celiac ganglia. Linking mechanisms between these different entities would interfere with the afferent limb of the great trophic circuit, either at the receptor level of vagal efferent fibers in the upper intestinal tract or at afferent pathways to the central nervous system from the resected pancreatic tissue. These phenomena suggest that partial excision of a diseased pancreatic gland, that is, disruption of the great trophic circuit, might induce pancreatic regeneration.

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In a previous communication, an unexpected increase in the weight of the body-tail segment of the pancreas after truncal vagotomy was reported (1). These vagotomy-induced changes were abolished by simultaneous chronic alcohol feeding (2). Further, mere sectioning and reanastomosis of the duodenal wall above and below the bile duct entry into the duodenum triggered an increase in the weight of the cephalic segment of the gland (2). On the other hand, bilateral splanchnicectomy prevented the vagotomy-induced pancreatic weight augmentation (unpublished observations).

These changes suggested that there are great and small trophic nervous reflex arcs centered about the pancreas. In the great trophic circuit, the afferent and efferent limbs would be the vagus and splanchnic nerves. Following disruption of the circuit by vagotomy, an escape of trophic impulses (via the spinal cord, the splanchnic nerves, and celiac ganglia) from the trophic center in the central nervous system appears to influence primarily the pancreatic body-tail segment. In the small trophic circuit, with less distinct anatomical connections, the organs involved would be the duodenum, the gastric antrum, and the celiac plexus. Its disruption by duodenotomy and long-term celiac ganglionectomy would produce the escape of trophic impulses, preferentially to the pancreatic head. Finally, chronic ethanol administration induces release from the central nervous system of antitrophic impulses that inhibit vagotomy-evoked pancreatic weight changes in the pancreatic body-tail segment (2).

This study was planned to corroborate these hypotheses. The protocol was designed to observe the pancreatic weight changes after disrupting either the great or the small trophic circuit. Chronic ethanol intoxication was used in some experiments to demonstrate its antitrophic activity.

Methods

All experiments, except those with pilocarpine, were performed on adult male Wistar rats. In total, 297 rats were used. The animals were fed a balanced diet (Purina, Buenos Aires) supplied ad libitum. Six control series (88 rats) comprised animals of different body weights. The experimental group consisted of 18 series (209 animals), studied sequentially following different surgical procedures designed to interrupt specific nervous arcs.

The animals were killed under ether anesthesia. The pancreas was carefully dissected from the surrounding tissues (lymph nodes and the surrounding fat tissue were removed). The head of the organ was isolated from the body and tail, the head being defined as the segment of the gland between the pylorus, the internal border of the duodenum, and a line drawn along the right border of the superior mesenteric vein and passing through the pylorus. The remaining gland was considered body-tail. The pancreatic parenchyma was blotted dry and weighed in a precision balance.

Two indices were analyzed: the ratio gm/kg (total weight of gland in grams, divided by body weight in kilograms), and the ratio BT/H (weight of body-tail segment compared to that of the head).

Statistical evaluation was by Student's *t* test for nonpaired values (3).

Control Series

The six control series included five groups of male rats of different weights and one of female rats; the latter served as a basis of comparison for evaluating the effect of chronic pilocarpine administration. The range of body weight of the six control series was 234–609 gm (Table 1).

Experimental Series

Truncal Vagotomy. Nineteen animals (body weight 391 ± 15.52 gm) were subjected to truncal vagotomy. After entering the abdomen through a midline incision, both the anterior and posterior vagus nerves were cut at the level of the distal esophagus using a dissecting microscope (1). Three months later, the animals were sacrificed. Body and pancreatic weights were determined (Table 2). Truncal vagisection was done on 14 additional animals (body weight 616 ± 33.8 gm) and weight data was obtained 16 months later (Table 3). Another 8 animals (body weight 379 ± 19.45 gm) had truncal vagotomy, and, immediately after the operation, were subjected to chronic alcohol feeding for 3 months. The rats were allowed to drink only a solution of ethyl alcohol according to the following schedule (4, 5): first week, a 5% solution (vol/vol); next two weeks, 10%; following week, 15%; fifth week,

TABLE 1

Weight Parameters in Controls

Weight	Female	Male				
Whole Body (gr)	234 ± 59	357 ± 5.3	385 ± 12.7	444 ± 6.3	573 ± 9.9	609 ± 6.1
Pancreas						
Head (mg)	384 ± 24.3	461 ± 18.8	499 ± 26.6	533 ± 21.8	643 ± 45.6	641 ± 22.0
Body-tail (mg)	772 ± 38.7	685 ± 29.1	835 ± 48.0	745 ± 29.0	926 ± 52.7	940 ± 49.8
TOTAL (mg)	1156 ± 54.9	1149 ± 90.2	1334 ± 67.2	1270 ± 241	1568 ± 83.8	1581 ± 69.1
Pancreas/ body weight (g/kg)	4.95 ± 0.21	3.2 ± 0.09	3.47 ± 0.16	2.87 ± 0.09	2.75 ± 0.16	2.60 ± 0.09
Body-tail/ head	2.04 ± 0.13	1.56 ± 0.009	1.70 ± 0.08	1.45 ± 0.09	1.50 ± 0.098	1.46 ± 0.04

dose increased to 25%. Afterward, the rodents received a solution of 32% alcohol. Weight data was obtained at 3 months (Table 4).

Bilateral Splanchnicectomy. Ten animals (body weight 438 ± 17.87 gm) had bilateral splanchnicectomy done by section of both splanchnic nerves at the level of their entry into the celiac plexus. Weighing of the pancreas was performed three months later (Table 5). Vagisection plus bilateral splanchnicectomy was performed on 14 animals (body weight 439 ± 17.8 gm) through a midline abdominal incision. Three months later the animals were sacrificed and weight measurements made (Table 6).

TABLE 2

Changes in Pancreatic Weight Induced by Short-Term (3-month) Truncal Vagotomy

Weight	Control (N = 28)	Experimental (N = 19)	Difference	p
Whole Body (gr)	357 ± 5.26	391 ± 15.52		
Pancreas				
Head (mg)	461 ± 18.8	604 ± 69	+31%	<0.02
Body-tail (mg)	685 ± 29.1	1322 ± 100	+93%	<0.001
TOTAL (mg)	1149 ± 90.2	1911 ± 115.5	+66%	<0.001
Pancreas/ body weight (g/kg)	3.2 ± 0.09	5.0 ± 0.32	+55%	<0.001
Body-tail/ head	1.56 ± 0.093	2.49 ± 0.32	+60%	<0.01

TABLE 3

Changes in Pancreatic Weight Induced by Long-Term (16-month) Truncal Vagotomy

Weight	Control (N = 15)	Experimental (N = 14)	Difference	p
Whole Body (gr)	573 ± 9.88	616 ± 33.8		
Pancreas				
Head (mg)	643 ± 45.59	717 ± 36.94	+13%	N.S.
Body-tail (mg)	926 ± 52.7	997 ± 107	+7%	N.S.
TOTAL (mg)	1568 ± 83.8	1721 ± 134	+8%	N.S.
Pancreas/ body weight (g/kg)	2.75 ± 0.16	2.79 ± 0.16	+7%	N.S.
Body-tail/ head	1.50 ± 0.098	1.37 ± 0.11	−11%	N.S.

Celiac Ganglionectomy. Celiac ganglionectomy was done on 23 animals (body weight 436 ± 16.40 gm). The abdomen was entered through a left paramedian incision. By retraction of the stomach to the right of the midline, the celiac and the superior mesenteric arteries could be identified. The mesentery between the arteries was divided at the level of their first branches. The area bounded by this incision, the aorta, and the two arteries contains the ganglia to be removed (6). Macroscopically, this region is easily recognized, since it has a much denser structure than the surrounding mesentery. The periarterial nerves distal to the ganglia were dissected free and cut, and the periarterial tissues, including the root of the mesentery, were stripped from the arteries down to the aorta. The aorta, too, was stripped in the zone between the origin of the celiac and superior mesenteric arteries. The ganglion-containing tissue could then be removed en bloc. Care was taken not to injure the lymphatic vessels that run close to the superior mesenteric artery. The entire procedure was performed under the dissecting microscope. The weight data was obtained at three months (Table 7). In an additional 13 rodents (body weight 444 ± 16.5 gm), surgical resection of the celiac plexus was imme-

TABLE 4

Short-Term Truncal Vagotomy plus Chronic Alcohol Feeding: Results after 3 months

Weight	Control (N = 28)	Experimental (N = 8)	Difference	p
Whole				
Body (gr)	357 ± 5.3	379 ± 19.4		
Pancreas				
Head (mg)	461 ± 18.8	377 ± 36.9	−18.2%	<0.05
Body-tail (mg)	685 ± 29.1	544 ± 48.3	−20.6%	<0.001
TOTAL (mg)	1149 ± 0.19	921 ± 66.5	−19.8%	<0.01
Pancreas/ body weight (g/kg)	3.22 ± 0.097	2.44 ± 0.53	−24.22%	<0.001
Body-tail/ head	1.56 ± 0.093	1.51 ± 0.18	−3.2%	N.S.

TABLE 5

Bilateral Splanchnicectomy: Results after 3 months

Weight	Control (N = 20)	Experimental (N = 10)	Difference	p
Whole				
Body (gr)	444 ± 6.3	438 ± 17.9		
Pancreas				
Head (mg)	533 ± 21.8	529 ± 46.5	−0.8%	N.S.
Body-tail (mg)	745 ± 29.0	707 ± 73.7	−5%	N.S.
TOTAL (mg)	1270 ± 36.2	1236 ± 111.8	3%	N.S.
Pancreas/ body weight (g/kg)	2.87 ± 0.09	2.80 ± 0.20	−2%	N.S.
Body-tail/ head	1.45 ± 0.09	1.37 ± 0.13	−6%	N.S.

TABLE 6

Truncal Vagotomy plus Bilateral Splanchnicectomy: Results after 3 months

Weight	Control (N = 20)	Experimental (N = 14)	Difference	p
Whole				
Body (gr)	444 ± 6.3	439 ± 17.8		
Pancreas				
Head (mg)	533 ± 21.8	625 ± 37.7	+17%	<0.05
Body-tail (mg)	745 ± 29.0	769 ± 66.7	+9%	N.S.
TOTAL (mg)	1270 ± 36.2	1393 ± 89.1	+10%	N.S.
Pancreas/ body weight (g/kg)	2.87 ± 0.09	3.22 ± 0.2	+12%	N.S.
Body-tail/ head	1.45 ± 0.09	1.25 ± 0.27	−13%	N.S.

diately followed by alcohol feeding for 3 months on the schedule previously described. Weight data is given in Table 8. Eleven other celiac ganglionectomized rats (body weight 728 ± 19.27 gm) were killed after 20 months (Table 9). Truncal vagisection was combined with celiac ganglionectomy in 15 animals (body weight 384 ± 14.64 gm) and weight data obtained after 3 months (Table 10). Five additional rodents (body weight 373 ± 21.27 gm) were alcohol fed for 3 months immediately after truncal vagotomy plus celiac ganglionectomy. Autopsy and weighing was performed three months later (Table 11).

Chronic Alcohol Feeding. Fifteen animals (body weight 417 ± 18.84 gm) were subjected to chronic ethanol intoxication for a 3-month period, after which they were killed and weight data obtained (Table 12).

Bowel Transection Experiments. In 6 young rats (body weight 376 ± 18.5 gm), the duodenum was sectioned and reanastomed just proximal and distal to the entry of the bile duct. Three months later, pancreatic weights were evaluated (Table 13). In 15 other older rats (body weight 504 ± 25.3 gm), a similar protocol was followed (Table 14).

Thirteen animals (body weight 444 ± 26.13 gm) had transection and reanastomosis of the pylorus. In 9 additional rodents (body weight 448 ± 15.53 gm), the transection and anastomosis were through the fundic-antral junction. The three-month weight data is given in Tables 15, 16.

Transection and reanastomosis of the duodenum proximal and distal to the bile duct entry, in addition to excision of the proximal duodenum, was performed in 7 animals (body weight 541 ± 22.84 gm). In an additional 6 rodents (body weight 508 ± 25.99 gm), this transection and duodenal anastomosis was carried out in conjunction with excision of the gastric antrum and proximal duodenum. The three-month data are shown in Tables 17, 18.

Pilocarpine Administration. The final protocol of the study involved subcutaneous daily pilocarpine (1.0 mg/kg) given to 6 female rats (body weight 228 ± 5.95 gm) for 10 days. At the end of this period, the animals were killed and weights determined (Table 19).

Results

Short-term vagotomy (3 months) significantly increased pancreatic weight compared to weights in the control series. Both the head (+31%) and body-tail segments (+39%) were augmented (Table 2, Fig. 1). After long-term vagotomy (16 months), there were no significant changes in pancreatic weights (Table 3). Alcohol feeding coupled with short-term truncal vagotomy completely blocked the trophic changes noted after vagal section (Table 4, Fig. 2). Values fell significantly below those in control studies.

Bilateral splanchnicectomy induced modifications of pancreatic weight (Table 5, Fig. 3). Bilateral splanchnicectomy associated with short-term truncal vagotomy completely prevented the vagotomy-induced weight increase of the body-tail segment and partially prevented that of the cephalic segment. The latter, however, remained significantly augmented (+17%) compared to the controls (Table 6, Fig. 4).

Short-term (3-month) celiac ganglionectomy did not elicit significant modifications of pancreatic weight (Table 7). Alcohol feeding superimposed on short-term (3-month) ganglionectomy, on the other hand, induced an increase of weight of the pancreatic head (Table 8, Fig. 5). Long-term (20-month) celiac ganglionectomy evoked a significant augmentation of the pancreatic head weight (Table 9, Fig. 6). Celiac ganglionectomy prevented the trophic changes induced by short-term vagotomy (Table 10). In contradistinction to the results observed when splanchnicectomy was associated with truncal vagotomy (Table 6), augmentation of the pancreatic head weight was not observed when alcohol was fed to animals subjected to truncal vagotomy plus celiac ganglionectomy for three months (Table 11). There was a significant increase of the pancreatic weight of both the head (+21.5%) and the total pancreas (+16%). The results were different from those observed following chronic ethanol administration to control rats or to animals subjected either to truncal vagotomy or celiac ganglionectomy. Indeed, ethanol alone, administered for three months, induced no significant changes of the pancreatic weight in comparison to control animals (Table 12).

Section and reanastomosis of the duodenum above and below the bile duct entry into the duodenum triggered a rise in the weight of the head segment of the pancreatic gland. This change was greater in the series of old rats (+61%) (Table 14) than in young ones (+47%) (Table 13, Fig. 7). Section and reanastomosis of the pylorus resulted in significant augmentation of the weight of the pancreatic head (+28%) compared to the control series (Table 15, Fig. 8). Section and reanastomosis at the level of the fundic-antral junction did not elicit significant changes of pancreatic weight (Table 16, Fig. 9). Section and reanastomosis of the duodenum above and below the bile duct entry into the duodenum plus the resection of the proximal segment of the duodenum (duodenal autonomic nervous brain) evoked a significant enlargement of the pancreatic head (+74%) and a simultaneous inhibition of growth (−22%) of the pancreatic body-tail segment (Table 17, Fig. 10). Finally, section and reanastomosis of the duodenum above and below the bile duct entry into the duodenum, plus resection of both the proximal duodenum and the gastric antrum, resulted in no significant changes of pancreatic weight (Table 18, Fig 11).

TABLE 7

Short-Term (3-month) Celiac Ganglionectomy

Weight	Control (N = 20)	Experimental (N = 23)	Difference	*p*
Whole Body (gr)	444 ± 6.3	436 ± 16.4		
Pancreas				
Head (mg)	533 ± 21.8	578 ± 24.2	+8%	N.S.
Body-tail (mg)	745 ± 29.0	732 ± 58.2	−2%	N.S.
TOTAL (mg)	1270 ± 36.2	1309 ± 71.2	+3%	N.S.
Pancreas/ body weight (g/kg)	2.87 ± 0.09	3.09 ± 0.19	+8%	N.S.
Body-tail/ head	1.45 ± 0.09	1.28 ± 0.10	12%	N.S.

TABLE 8

Short-Term Celiac Ganglionectomy plus Alcohol Feeding: Results after 3 months

Weight	Control (N = 20)	Experimental (N = 13)	Difference	*p*
Whole Body (gr)	444 ± 6.3	444 ± 16.5		
Pancreas				
Head (mg)	533 ± 21.8	682 ± 47.8	+27.9%	<0.01
Body-tail (mg)	745 ± 29.0	894 ± 139.7	+20.0%	N.S.
TOTAL (mg)	1270 ± 241.4	1576 ± 173	+24.0%	<0.05
Pancreas/ body weight (g/kg)	2.87 ± 0.09	3.59 ± 0.33	+25.09%	<0.02
Body-tail/ head	1.45 ± 0.09	1.30 ± 0.12	−10.3%	N.S.

TABLE 9

Long-Term (20-month) Celiac Ganglionectomy

Weight	Control (N = 9)	Experimental (N = 11)	Difference	*p*
Whole Body (gr)	609 ± 6.14	728 ± 19.27		
Pancreas				
Head (mg)	641 ± 22.0	936 ± 74.9	+46%	<0.001
Body-tail (mg)	940 ± 49.8	826 ± 78.3	−12.12%	<0.001
TOTAL (mg)	1581 ± 69.09	1762 ± 85.7	+11.45%	N.S.
Pancreas/ body weight (g/kg)	2.60 ± 0.09	2.45 ± 0.13	−5.76%	N.S.
Body-tail/ head	1.46 ± 0.04	0.97 ± 0.13	−33.56%	<0.001

Administration of pilocarpine for 10 days induced a significant increase in the weight of the body-tail segment of the pancreas (+37%) but not of the pancreatic head (Table 19).

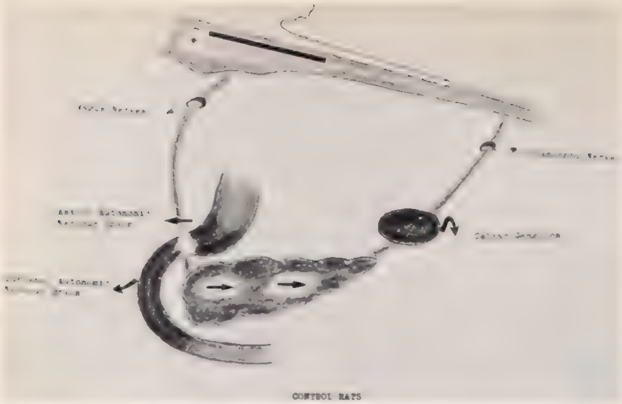
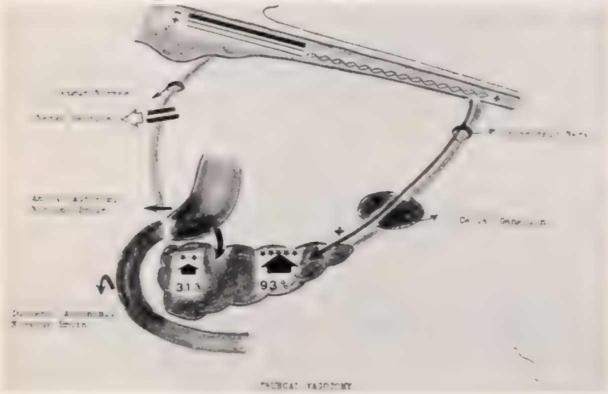

Vagus Nerves
Splanchnic Nerves
Antral Autonomic Nervous Brain
Celiac Ganglion
Duodenal Autonomic Nervous Brain
CONTROL RATS

Fig. 1. Left Main constituents of small trophic circuit (duodenum, gastric antrum, and celiac plexus) and great trophic circuit (vagus, afferent limb; splanchnic nerve, efferent limb). When they are in equilibrium, weight of pancreatic head and body-tail segments remain invariable, reflected in indexes g/kg and BT/H (control rats). Horizontal arrow (→), in this and other figures, indicates pancreatic weight not significantly different from control, either of head or body-tail segment.


Vagus Nerves
Vagal Section
Splanchnic Nerves
Antral Autonomic Nervous Brain
Celiac Ganglion
31%
93%
Duodenal Autonomic Nervous Brain
TRUNCAL VAGOTOMY

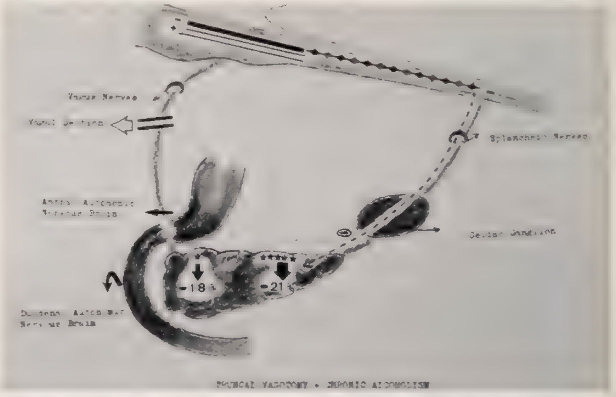
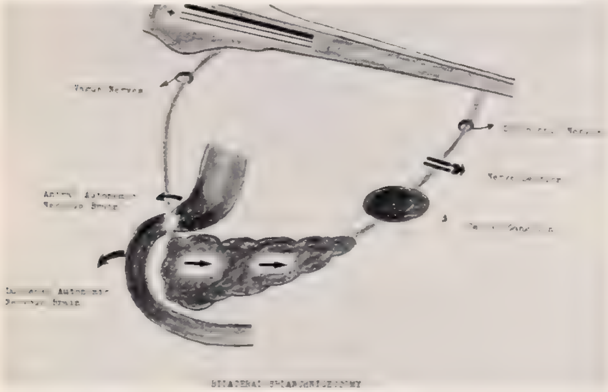

Vagus Nerves
Vagal Section
Splanchnic Nerves
Antral Autonomic Nervous Brain
Celiac Ganglion
-18%
-21%
Duodenal Autonomic Nervous Brain
TRUNCAL VAGOTOMY + CHRONIC ALCOHOLISM

Fig. 2. Left Short-term (3-month) truncal vagotomy induces imbalance of great trophic circuit. Trophic impulses from a hypothetical positive trophic center in central nervous system reach pancreatic gland, mainly body-tail segment, via spinal cord, splanchnic nerves, and celiac ganglia. Vertical arrow (↑) indicates pancreatic enlargement.

Fig. 3. Right Chronic alcohol feeding superimposed on vagotomy-induced imbalanced great trophic circuit triggers antitrophic impulses from central nervous system that not only block vagotomy-evoked pancreatic enlargement but reduce pancreatic weight to levels significantly below those of controls. Vertical arrow (↓) indicates pancreatic weight reduction below control level.


Vagus Nerves
Splanchnic Nerves
Nerve Section
Antral Autonomic Nervous Brain
Celiac Ganglion
Duodenal Autonomic Nervous Brain
BILATERAL SPLANCHNICECTOMY

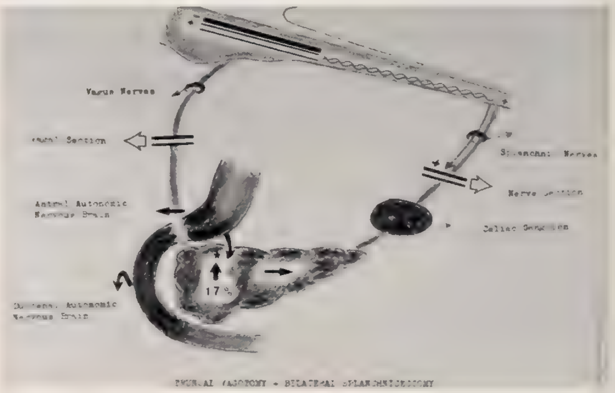

Vagus Nerves
Vagal Section
Splanchnic Nerves
Nerve Section
Antral Autonomic Nervous Brain
Celiac Ganglion
17%
Duodenal Autonomic Nervous Brain
TRUNCAL VAGOTOMY + BILATERAL SPLANCHNICECTOMY

Fig. 4. Left Bilateral splanchnicectomy (interruption of efferent limb of great trophic circuit) does not modify pancreatic weight, either of head or body-tail segment.

Fig. 5. Right Bilateral splanchnicectomy (interruption of efferent limb of great trophic circuit) completely prevents weight increase of body-tail segment and partially prevents that of cephalic segment, evoked by short-term truncal vagotomy.

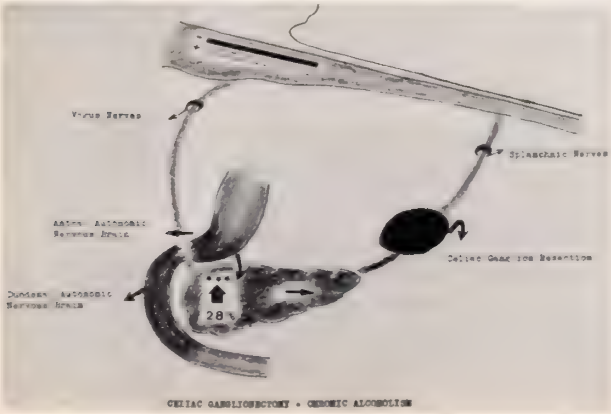
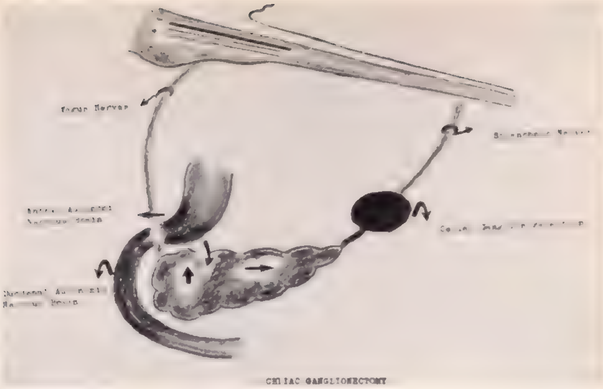
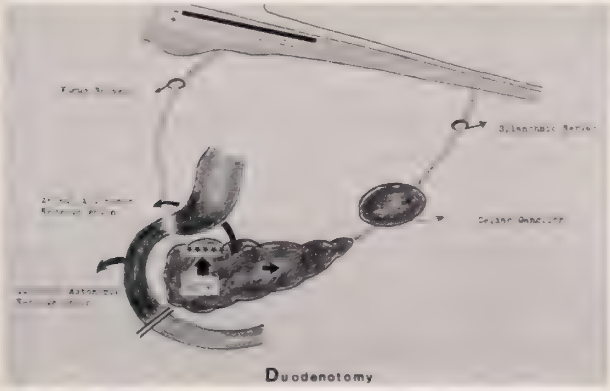

Vagus Nerves
Splanchnic Nerves
Antral Autonomic Nervous Brain
Celiac Ganglion Resection
Duodenal Autonomic Nervous Brain
28%
CELIAC GANGLIONECTOMY + CHRONIC ALCOHOLISM

Fig. 6. Left Alcohol feeding superimposed on short-term (3-month) celiac ganglionectomy (disruption of small trophic circuit) induces weight increase of pancreas head. Trophic impulse would be triggered by ethanol on stomach antrum (antral autonomic nervous brain) free of modulatory influence of celiac ganglia.

Fig. 7. Long-term (20-month) celiac ganglionectomy (disruption of small trophic circuit) elicits increase of pancreatic weight of head segment.


Vagus Nerves
CELIAC GANGLIONECTOMY


Celiac Ganglion
Duodenotomy

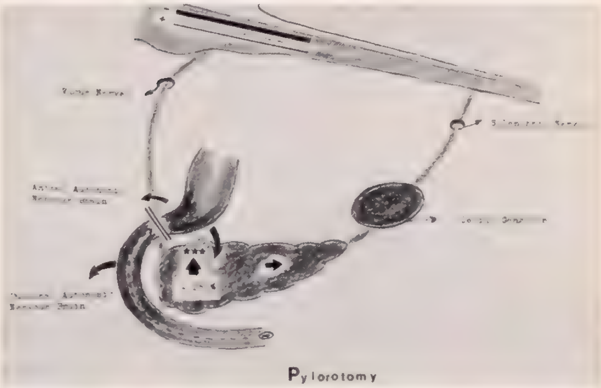
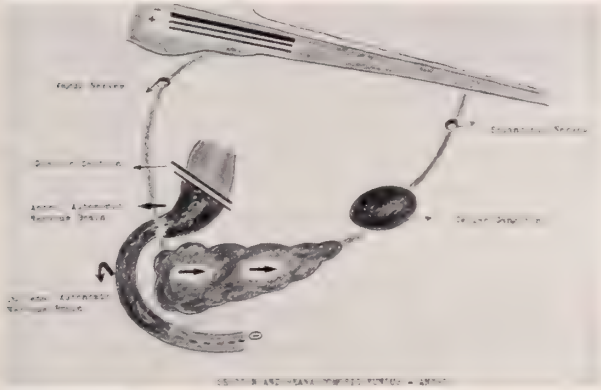

Vagus Nerves
Pylorotomy

Fig. 8. Left Section and reanastomosis of duodenal wall above and below bile duct entry into duodenum evokes marked increase of pancreatic head weight (disruption of small trophic circuit with stomach antrum—antral autonomic nervous brain—escape).

Fig. 9. Right Section and reanastomosis of the gut wall at the level of the pylorus (pylorotomy) evokes a significant pancreatic enlargement of the head segment (disruption of the small trophic circuit with antral autonomic nervous brain—escape).



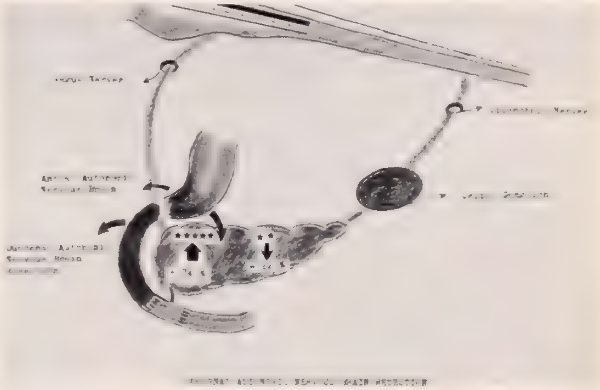


Fig. 10. Left Section and reanastomosis of the gut wall at the level of the fundus-antrum junction does not elicit pancreatic weight changes.

Fig. 11. Right Coupling of duodenotomy to proximal duodenal segment resection (duodenal autonomic nervous brain exeresis) induces marked enlargement of pancreatic head and simultaneous growth inhibition of pancreatic body-tail segment (disruption of small trophic circuit).

Fig. 12. Right Exeresis of antral autonomic nervous brain completely prevents pancreatic head enlargement and inhibition of growth of pancreatic body-tail segment induced by duodenotomy plus resection of duodenal autonomic nervous brain.

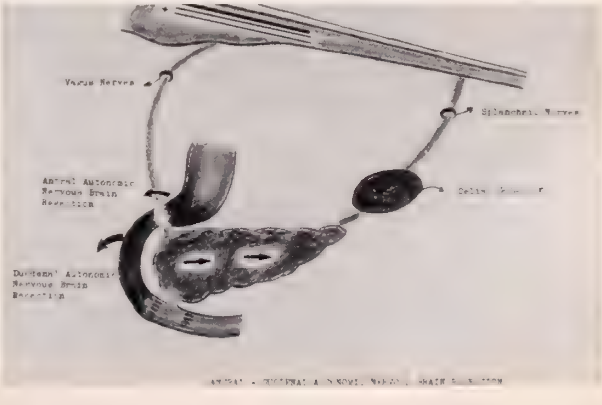

Vagus Nerves
Splanchnic Nerves
Antral Autonomic
Nervous Brain
Resection
Duodenal Autonomic
Nervous Brain
Resection

TABLE 10

Short-Term Truncal Vagotomy plus Celiac Ganglionectomy: Results after 3 months

Weight	Control (N = 10)	Experimental (N = 15)	Difference	*p*
Whole Body (gr)	385 ± 12.7	384 ± 14.6		
Pancreas				
Head (mg)	499 ± 26.6	548 ± 36.1	+10%	N.S.
Body-tail (mg)	835 ± 48.0	872 ± 72.6	+4%	N.S.
TOTAL (mg)	1334 ± 67.2	1420 ± 93.3	+6%	N.S.
Pancreas/ body weight (g/kg)	3.47 ± 0.16	3.71 ± 0.23	+7%	N.S.
Body-tail/ head	1.70 ± 0.08	1.62 ± 0.12	−5%	N.S.

TABLE 11

Short-Term Truncal Vagotomy plus Celiac Ganglionectomy plus Chronic Alcohol Feeding: Results after 3 months

Weight	Control (N = 28)	Experimental (N = 5)	Difference	*p*
Whole Body (gr)	357 ± 5.26	373 ± 21.17		
Pancreas				
Head (mg)	461 ± 18.8	560 ± 44.4	+21.5%	<0.05
Body-tail (mg)	685 ± 29.1	773 ± 48.1	+12.9%	N.S.
TOTAL (mg)	1149 ± 0.2	1333 ± 78.6	+16.0%	<0.05
Pancreas/ body weight (g/kg)	3.22 ± 0.09	3.57 ± 0.3	+10.9%	N.S.
Body-tail/ head	1.56 ± 0.09	1.40 ± 0.08	−10.3%	N.S.

TABLE 12

Chronic Alcohol Feeding (3–4 months)

Weight	Control (N = 20)	Experimental (N = 15)	Difference	*p*
Whole Body (gr)	444 ± 6.28	417 ± 18.8		
Pancreas				
Head (mg)	533 ± 21.7	531 ± 30.5	−0.4%	N.S.
Body-tail (mg)	745 ± 29.0	732 ± 56.5	−1.7%	N.S.
TOTAL (mg)	1270 ± 36.2	1288 ± 73.6	+1.4%	N.S.
Pancreas/ body weight (g/kg)	2.87 ± 0.09	3.22 ± 0.2	+12.2%	N.S.
Body-tail/ head	1.45 ± 0.09	1.37 ± 0.06	−5.52%	N.S.

Discussion

The present studies confirm our previous finding (1) that truncal vagotomy significantly increases the pancreatic wet weight. The change is more marked in the body-tail segment (+93%) than in the head of the organ (+31%). However, this pancreatic enlargement progressively regresses with time. Indeed, after a 16-month period, the differences compared with controls were effaced. On the other hand, splanchnicectomy, per se, did not elicit pancreatic weight changes; rather, it prevented the trophic changes induced by truncal vagotomy, except for a slight but significant increase of the weight of the head segment (+17%). These observations, coupled with the fact that celiac ganglionectomy also abolishes vagotomy-evoked pancreatic weight augmentation, supports the hypothesis that truncal vagotomy, by interruption of afferent pathways to the central nervous system, triggers the discharge of impulses from a positive trophic center (Fig. 1). These nervous impulses would reach the body-tail segment of the pancreas by passing through the spinal cord, the splanchnic nerves, and the celiac ganglia. Thus, trophic influences could be related to the adrenergic autonomic nervous system.

This speculation is an extrapolation to the pancreas of observations of the small intestine that have indicated that noradrenaline stimulates crypt cell proliferation in rat intestine by acting on alpha-adrenoceptors. Furthermore, in both jejunum and colon, the alpha-adrenergic antagonist, phentolamine, slows cell proliferation, while beta-adrenergic blockers lack this effect (7). On the other hand, in the salivary glands, beta receptor activation triggers a trophic response (8). The pancreatic trophic circuits bear striking resemblance to salivary trophic reflex arcs (cutting the chord lingua on one side results in enlargement of the submandibular gland on the other side). This change is blocked by simultaneous removal of the superior cervical ganglia on the hypertrophic side.

The hypothesis of vagal-sympathetic interaction in trophic arcs is further strengthened (9, 10) by data indicating cholinergic and/or peptinergic participation in trophic arcs in the rat jejunum, that is, stimulation of cholinergic receptors by either the cholinergic agonist carbachol, or by elevated levels of endogenous acetylcholine after treatment with the acetylcholinesterase inhibitor neostigmine, promotes crypt cell proliferation (11). In the pancreas, Mainz et al (12) did not find any changes with bethanechol. On the other hand, in rats, administration of carbachol or electrical vagal stimulation increases DNA synthesis rates (13). In our studies, an increase in the weight of the body-tail segment, but not in the head of the pancreas, was observed after pilocarpine injections (1.0 mg/kg for 10 days). This peculiar segmental response of the pancreas presumably results from cholinomimetic activation of a regulatory peptide in the celiac ganglia which, preferentially, exerts its influence upon the body-tail of the pancreatic gland. Furthermore, chronic administration of a muscarinic receptor blocker (atropine) has been reported to increase the secretory capacity of the pancreatic gland (14).

The concept of a short trophic circuit evolved from finding that duodenotomy (section and reanastomosis of the duodenal wall above and below the bile duct entry into the duodenum) triggered a marked increase in the weight of the cephalic segment of the pancreatic gland. The changes were more marked (+75%) in the aged animals than in young rats (+45%). The surgical procedure presumably interrupts inhibitory signals coming from the lower intestine, thus favoring escape of trophic impulses from the duodenal and antral autonomic nervous brains. In order to confirm this hypothesis, sectioning and reanastomosis of the gut wall was done at higher levels. When performed at the pylorus, the increase in weight (+28%) of the pancreatic head was less marked. When carried out at the fundic-antral junction, there were no pancreatic trophic changes. Therefore, the gastric fundus is not a significant source of trophic impulses to the pancreas.

A number of factors might be involved in the pathogenesis of the described pancreatic weight alteration. One might be the level of plasma secretin, since a rise in blood secretin has been reported following resection of the proximal rat small intestine (15). Yet secretin, per se, cannot account for the massive pancreatic weight changes encountered. Secretin, at best, has a weak pancreatotrophic capacity. On the other hand, secretin is a strong potentiator of cholecystokinin-pancreozymin (CCK-PZ) activity (16, 17). In fact, CCK-PZ, gastrin, and bombesin (GRP) are known potent pancreatrophic agents. These hormones, which are released after food intake, produce cellular hypertrophy and hyperplasia in the rat pancreas (16–18). Furthermore, increases of CCK-PZ in plasma after duodenal diversion of bile-pancreatic juice or after ingestion or infusion of trypsin inhibitors (19, 20) also induce rodent pancreatic enlargement. In addition, it has been reported that CCK-PZ and GRP can trigger by a paracrine-neural mechanism duodeno-pancreatic reflexes (short, medium, and long) stimulating the "pancreon" units either directly or indirectly via intrapancreatic ganglia (21–23); this mechanism operates independently of the hormone's blood levels. Thus, after duodenotomy, there is an interruption of impulses coming from the lower segments of the intestinal tract, impulses normally modulating the activity of both the duodenal and antral autonomic nervous brains. Indeed, several reports confirm that the lower intestine exerts influences upon the pancreas and gallbladder by means of a neuro-endocrine mechanism (24–26). Hence, following duodenotomy, there is an escape of trophic impulses to the pancreas from the duodenal and antral autonomic nervous brains (27–29). After pylorotomy, the antrum would become an auto-

TABLE 13

Duodenotomy in Young Rats: Results after 3 months

Weight	Control (N = 28)	Experimental (N = 6)	Difference	*p*
Whole				
Body (gr)	357 ± 5.3	376 ± 18.5		
Pancreas				
Head (mg)	461 ± 18.8	676 ± 15.5	+47%	<0.001
Body-tail (mg)	685 ± 29.1	773 ± 16.7	+13%	N.S.
TOTAL (mg)	1149 ± 90.1	1486 ± 28.1	+29%	<0.001
Pancreas/ body weight (g/kg)	3.22 ± 0.09	3.99 ± 0.14	+24%	<0.01
Body-tail/ head	1.56 ± 0.09	1.15 ± 0.04	−26%	N.S.

TABLE 14

Duodenotomy in Old Rats: Results after 3 months

Weight	Control (N = 15)	Experimental (N = 15)	Difference	*p*
Whole				
Body (gr)	573 ± 9.9	504 ± 45.6		
Pancreas				
Head (mg)	643 ± 45.6	1033 ± 67.9	+61%	<0.001
Body-tail (mg)	926 ± 52.7	1211 ± 142	+31%	N.S.
TOTAL (mg)	1568 ± 83.8	2244 ± 155	+43%	N.S.
Pancreas/ body weight (g/kg)	2.75 ± 0.16	4.58 ± 0.37	+66%	<0.001
Body-tail/ head	1.50 ± 0.098	1.27 ± 0.22	−15%	N.S.

TABLE 15

Pylorotomy: Results after 3 months

Weight	Control (N = 20)	Experimental (N = 13)	Difference	*p*
Whole				
Body (gr)	444 ± 6.3	444 ± 26.1		
Pancreas				
Head (mg)	533 ± 21.8	683 ± 43.4	+28%	<0.01
Body-tail (mg)	745 ± 29.0	739 ± 82.7	−0.1%	N.S.
TOTAL (mg)	1270 ± 36.2	1422 ± 112	+12%	N.S.
Pancreas/ body weight (g/kg)	2.87 ± 0.09	3.26 ± 0.24	+14%	N.S.
Body-tail/ head	1.45 ± 0.09	1.09 ± 0.1	−24%	N.S.

TABLE 16
Pyloric Section and Reanastomosis at Fundic-Antrae Junction: Results after 3 months

Weight	Control (N = 20)	Experimental (N = 9)	Difference	p
Whole				
Body (gr)	444 ± 6.3	448 ± 13.5		
Pancreas				
Head (mg)	533 ± 21.8	527 ± 28.4	−1%	N.S.
Body-tail (mg)	745 ± 29.0	787 ± 58.6	+6%	N.S.
TOTAL (mg)	1270 ± 36.2	1314 ± 60.6	+3%	N.S.
Pancreas/ body weight (g/kg)	2.87 ± 0.09	2.85 ± 0.23	−0.7%	N.S.
Body-tail/ head	1.45 ± 0.09	1.67 ± 0.23	+15%	N.S.

TABLE 17
Duodenotomy plus Resection of the Duodenal Autonomic Nervous Brain: Results after 3 months

Weight	Control (N = 15)	Experimental (N = 7)	Difference	p
Whole				
Body (gr)	573 ± 9.9	541 ± 22.8		
Pancreas				
Head (mg)	643 ± 45.6	1122 ± 108	+74%	<0.001
Body-tail (mg)	926 ± 52.9	724 ± 24.5	−22%	<0.025
TOTAL (mg)	1568 ± 83.8	1847 ± 114	+18%	N.S.
Pancreas/ body weight (g/kg)	2.75 ± 0.16	3.46 ± 0.29	+26%	<0.05
Body-tail/ head	1.50 ± 0.098	0.68 ± 0.06	−55%	<0.001

TABLE 18
Duodenotomy plus Resection of the Duodenal and Antral Autonomic Nervous Brains: Results after 3 months

Weight	Control (N = 13)	Experimental (N = 6)	Difference	p
Whole				
Body (gr)	573 ± 9.9	508 ± 25.9		
Pancreas				
Head (mg)	643 ± 45.6	752 ± 41.13	+17%	N.S.
Body-tail (mg)	926 ± 52.7	725 ± 96.9	−21.7%	N.S.
TOTAL (mg)	1568 ± 83.8	1477 ± 121.5	−5.8%	N.S.
Pancreas/ body weight (g/kg)	2.75 ± 0.16	2.93 ± 0.24	+6.5%	N.S.
Body-tail/ head	1.50 ± 0.098	0.96 ± 0.12	−36%	<0.01

nomic nervous brain free from distal restraining influences.

That the antral segment of the stomach is an important neuro-endocrine center modulating pancreatic and cholecystic activity has been reported in several studies (30, 31). The duodenotomy procedures (section and reanastomosis of the duodenal wall above and below the bile duct entry into the duodenum), followed either by the resection of the proximal duodenum (Fig. 10) or extirpation of the duodenal and antral autonomic nervous brains (Fig. 11), further elucidate the arcs of the small-circuit trophic reflexes. The former procedure produced a marked increase in the weight of the cephalic pancreatic segment (+74%). On the other hand, when both the proximal duodenum and the gastric antrum were removed, the pancreatic weight remained unchanged. The latter finding indicates that disconnection of the antrum from the rest of the intestinal tract (duodenotomy, pylorotomy, proximal duodenal resection) unmasks its potential of discharging trophic impulses to the pancreatic head. However, the effects of antrectomy, per se, on the pancreas are controversial. One study reports that excision of the antrum, which would lower circulating blood gastrin, has had little effect on pancreatic DNA synthesis (32). Another report states that this resection results in pancreatic atrophy, without distinction between the head and body-tail segments (33). These latter results were confirmed by Oscarson et al (34) in rodents with antrectomy done with Billroth's operation I, but not when gastro-intestinal reconstruction was done with Billroth's operation II.

In our studies, the antrum escaped from restraining influences when either the vagus nerves or the celiac ganglia were cut. Thus, following truncal vagotomy, there was a slight weight increase in the pancreatic head and a marked augmentation in the weight of body-tail segment. The latter change, but not the former, was abolished by coupling bilateral splanchnicectomy with truncal vagotomy. In addition, following long-term celiac ganglionectomy, there was an increase in the weight of the pancreatic head (+46%). Therefore, both the vagus nerves and the celiac ganglia exert a restraining modulatory influence upon the antrum.

The question as to the nature of trophic impulses in the long and short trophic circuits needs clarification. It seems unlikely that the agents involved could be the same for both circuits. The data suggest that impulses acting on the body-tail segment are controlled by adrenergic impulses coming from the central nervous system and passing through the spinal cord, splanchnic nerves, and celiac ganglia. The pancreatic head, on the other hand, is under peptidergic influences coming from the gastric antrum.

The present results indicate a marked antitrophic effect of alcohol on the pancreas, particularly when superimposed on previous vagisection. These findings are consistent with the observations of Wormsley et al (35), who noted that alcohol feeding blocked pancreatic regeneration in rats after partial pancreatectomy. While alcohol feeding, per se, did not affect pancreatic weight (4), alcohol intoxication in vagotomized rats was antitrophic, which implies interruption of the afferent limb of the great trophic circuit, allowing escape of alcohol-induced antitrophic impulses from hypothalamic-bulbar centers. These impulses would reach the pancreas by a long efferent loop via the spinal cord, the splanchnic nerves, and the celiac ganglia. Finally, chronic alcohol feeding, superimposed on the totally decentralized pancreatic gland (truncal vagotomy plus celiac ganglionectomy), triggered significant enhancement of the pancreatic weight both in the head (+21.5%) and the total pancreas (+16%).

TABLE 19

Chronic Administration of Pilocarpine (1.0 mg/kg for 10 days) to Female Rats

Weight	Control (N = 6)	Experimental (N = 6)	Difference	p
Whole Body (gr)	234 ± 5.9	228 ± 5.9		
Pancreas				
Head (mg)	384 ± 24.3	428 ± 24.5	+11.5%	N.S.
Body-tail (mg)	772 ± 38.7	1057 ± 34.1	+37%	<0.001
TOTAL (mg)	1156 ± 54.9	1484 ± 48.9	+28.4%	<0.005
Pancreas/ body weight (g/kg)	4.95 ± 0.21	6.58 ± 0.10	+31.7%	<0.001
Body-tail/ head	2.04 ± 0.13	2.52 ± 0.16	+23.5%	<0.05

The pancreatotrophic action of chronic administration of trypsin inhibitors to the rat is intriguing. Melmed and Bouchier (36) suggested that the pancreatic enlargement observed in the rat following chronic administration of raw soybean was mediated through the vagus nerves. However, after unilateral cervical vagotomy, they observed no pancreatic weight increase. Our finding following bilateral vagisection (1) confirmed their hypothesis. Further support derives from the results observed by Ihse et al in rats (37). They reported that intraduodenal infusion of trypsin inhibitors, in the presence of bile-pancreatic juice or trypsin, elicited an increase of basal bile-pancreatic protein secretion, an effect that was prevented by acute truncal vagotomy, but not by an anticholinergic. Furthermore, it was shown that an intraduodenal infusion of trypsin abolishes the protein hypersecretion triggered by the diversion from the duodenum of bile-pancreatic juice (38). These phenomena can be interrupted as follows: the trypsin inhibitors, acting at the level of the upper intestine, prevent trypsin-induced discharge of afferent impulses to the central nervous system through the vagus nerves, an effect equivalent to surgically selective interruption of vagal afferent fibers. Since anticholinergics, such as probanthine, are unable to abolish the trypsin inhibitor induced bile-pancreatic hypersecretion, there must result an escape of increased efferent secretory discharge via either the vagus or the splanchnic nerves.

Finally, confirming the concept of trophic circuits are the reports that chronic diversion of pancreatic enzymes from the rat duodenum (39), chronic biliary diversion to the urinary tract, and feeding cholestyramine, an anion-exchange resin binding luminal bile salts (40), each elicit pancreatic growth. We hold these phenomena to be equivalent to chronic vagisection, in the sense that all induce disruption of the great trophic circuit by interfering with its afferent limb. Others (19, 20) have interpreted the data to result from increased serum CCK-PZ. These interpretations are not mutually exclusive; in fact, they are likely to be complementary—witness the opinion of Wood et al (39) that trophic changes of the pancreas and small intestine could not be totally explained by raised blood CCK-PZ level.

Conceptually, we would also equate partial removal of the pancreas to interfering with the afferent limb of the great trophic circuit by truncal vagotomy. The regeneration phenomenon observed under these circumstances (44, 45) would be due to an increased discharge of trophic impulses from the central nervous system via the spinal cord, splanchnic nerves, and the celiac ganglia (12). That this analogy is reasonable is supported by the fact that the trophic response of the pancreatic gland elicited by its partial resection, as in the case of truncal vagotomy and the feeding of trypsin inhibitors (41), is abolished by superimposed chronic administration of ethanol. That the pancreatic gland has a marked regenerative capacity was shown by Dreiling et al (46, 47), who triggered regeneration by partial resection of diseased parenchyma in the dog as well as in humans (48). Indeed, sequential secretin tests for several years after left subtotal pancreatectomy in patients with chronic alcoholic pancreatitis showed progressive restoration of pancreatic secretion, even beyond presurgical levels, but only in patients who had ceased drinking. Thus, the concept of trophic circuits governing the pancreatic mass has not only physiopathogenic but also therapeutic implications.

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Estrogen Effects on Exocrine Pancreatic Secretion in Menopausal Women: A Hypothesis for Menopause-Induced Chronic Pancreatitis

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Abstract

Estradiol valerianate (4 mg/day) administered to menopausal women alters secretin-evoked pancreatic secretory responses. Estrogens given for short periods (one month) trigger augmentation of exocrine pancreatic secretion. All secretin-induced parameters (flow, bicarbonate, and protein) show a significant increase in respect to their pre-estrogen levels. Estrogen administered for long periods (three to four months) induces significant decreases of secretin-induced pancreatic secretion. The excitatory phase (brief estrogen administration) results from activitation of the cytosolic receptors of the pancreon (the pancreas secreting unit—ducts as well as acinar tissue). This enhances the normal potentiating interplay between estrogen and secretin receptors in acinar and ductular cell membranes. This mechanism also explains the postsecretin hypersecretion observed in patients with alcoholic hepatic cirrhosis. The inhibitory phase (prolonged estrogen administration) is the consequence of a desensitization phenomenon (estrogen receptor down-regulation) and a lessening of the normal potentiating interaction between the receptors involved. In one patient, reversal to pre-estrogen values was observed two months after cessation of prolonged (four-month) estradiol administration. The decline of plasma estradiol in menopausal women thus could play a role in the pathogenesis of chronic pancreatitis observed during the menopause. The mechanism would be impairment of the normal interplay between the pancreatic estrogenic tone and those of the pancreon secretin receptors, resulting in a significant decrease of the hydrelatic component of secretin-induced pancreatic secretion, an effect favoring intraductal precipitation of protein plugs.

Previous reports (1–3) have shown that post-secretin exocrine pancreatic secretion is significantly lower in women over 45 years of age than in men of the same age. There is data indicating that the decrease in plasma estradiol during the menopause (4) induces this alteration of pancreatic secretion and is related to modification of activity of estrogen receptors found in both the exocrine and the endocrine pancreas (5, 6). Moreover, parenteral administration of estradiol benzoate to male rats increases the hydrelatic (water and electrolyte secreting) as well as the ecbolic component of basal bile-pancreatic secretion (7).

This study was designed to evaluate the effect of the administration of estradiol to menopausal women on secretin-induced pancreatic secretion.

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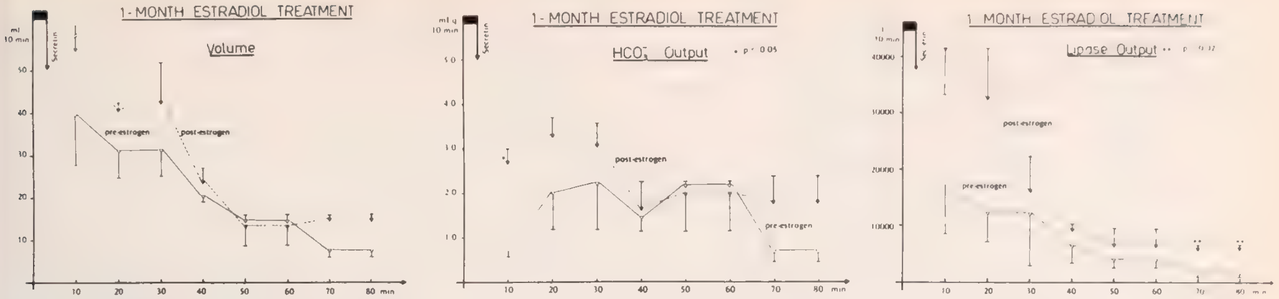

Fig. 1. Postsecretin secretory pattern (volume and bicarbonate and lipase output) of bile-pancreatic secretion before and after short-term (1 month) estrogenic treatment.

Patients, Materials, and Methods

In the protocol, each subject acted as her own control. Thus, in eleven menopausal women (mean age, 50.5 ± 1.18; range, 45–57) free of digestive disorder, two secretin tests were performed, one prior to estradiol administration and the second during estrogen administration. In three women, estradiol valerianate was administered at the dose of 4 mg/day for one month. In eight women, the same dose of estrogen was given for three to four months. In one woman, three tests were carried out: one before, a second during, and a third after cessation of estrogen therapy.

In the secretin test (Boots; 1.0 U/kg, intravenous bolus injection) two types of analysis were performed. First, the secretory patterns of flow and of bicarbonate and protein concentration and output for each of eight 10-minute collections were charted. Second, 80-minute cumulative values (8–10) were measured.

The measurements were as follows: volume, ml/10 min, ml/80 min; bicarbonate concentration, mEq/1, by the titrimetric method with nitric acid of Lehmann et al; bicarbonate output, mEq/10 min, mEq/80 min; lipase concentration, U/ml, by the method of Lehmann et al (12); lipase output, U/10 min, U/80 min, U/kg; amylase concentration, U/ml, determined in the 80-min pool, following the Somogyi method (8, 9); amylase output, U/80 min, U/kg.

Statistical analysis was performed by Student's *t* test for nonpaired values (13); $p < 0.05$ was considered significant.

Results

Estrogen Administration, One Month. The secretory pattern (eight periods, 10 min each) before and during estradiol therapy (Fig. 1) discloses the estradiol induced initial significant increases in flow and bicarbonate and lipase output. The analysis of 80 min cumulative data (Fig. 2) reveals a nonsignificant posttreatment augmentation of volume, a significant increase in bicarbonate output, and a statistically significant augmentation of lipase output.

Estrogen Administration, 3–4 Months. The data comparing secretory patterns before and following prolonged administration of estradiol (Fig. 3) show that estradiol treatment induces significant and prolonged decrease in flow. Bicarbonate concentrations are the same before and after estradiol, but bicarbonate output is significantly diminished. Lipase concentrations are lower but not significantly decreased throughout the 80-minute collection periods. On the other hand, postsecretin lipase output following estradiol administration is significantly inhibited.

The 80-minute cumulative data before and during estradiol administration (Fig. 4) show that estradiol induced a significant decline in flow and bicarbonate output. Both lipase and amylase output in response to secretin diminished after prolonged administration of estradiol, but only the fall in amylase is statistically significant.

In one healthy menopausal woman, three secretin tests (Fig. 5) were performed: one pre-estrogen, a second one after 4 months of estradiol administration, and a third test 2 months after discontinuation of estradiol. After prolonged estradiol administration, flow and bicarbonate and lipase output show the expected inhibition of secretin-stimulated secretion. Two months after cessation of estradiol, levels of secretion return to and overshoot pre-estradiol levels.

Discussion

The data disclose that estradiol administered to menopausal women elicits a biphasic response. Initially, there is excitation of both the hydrelatic and ecbolic components of the postsecretin stimulated pancreatic secretion (Figs. 1, 2); the second phase is inhibition (Figs. 3, 4).

In a previous study, we have reported signifi-

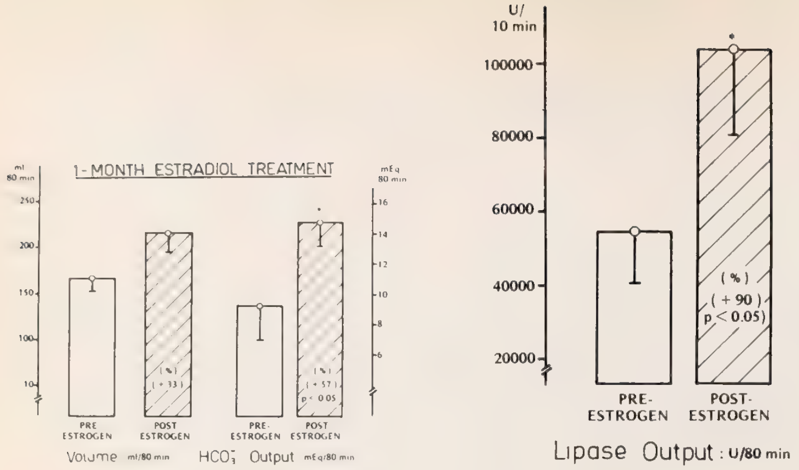

Fig. 2. Left Postsecretin 80-min cumulative values (volume and bicarbonate and lipase output) of bile-pancreatic secretion before and after short-term (1 month) estrogenic treatment.

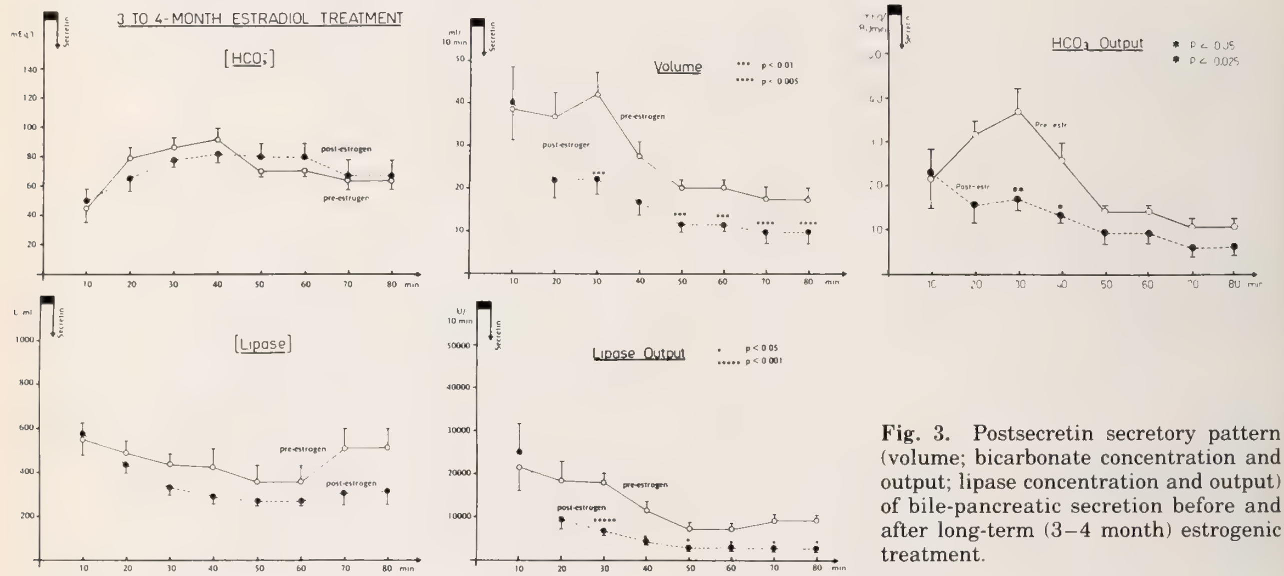

Fig. 3. Postsecretin secretory pattern (volume; bicarbonate concentration and output; lipase concentration and output) of bile-pancreatic secretion before and after long-term (3–4 month) estrogenic treatment.

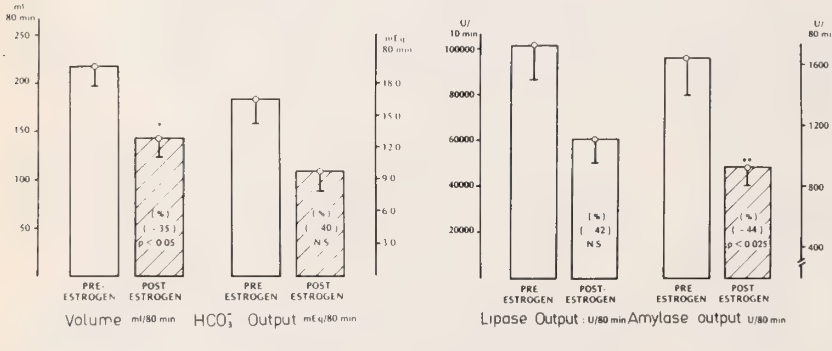

Fig. 4. Postsecretin 80-min cumulative values (volume; output of bicarbonate, lipase, and amylase) of bile-pancreatic secretion before and after long-term (3–4 month) estradiol treatment.

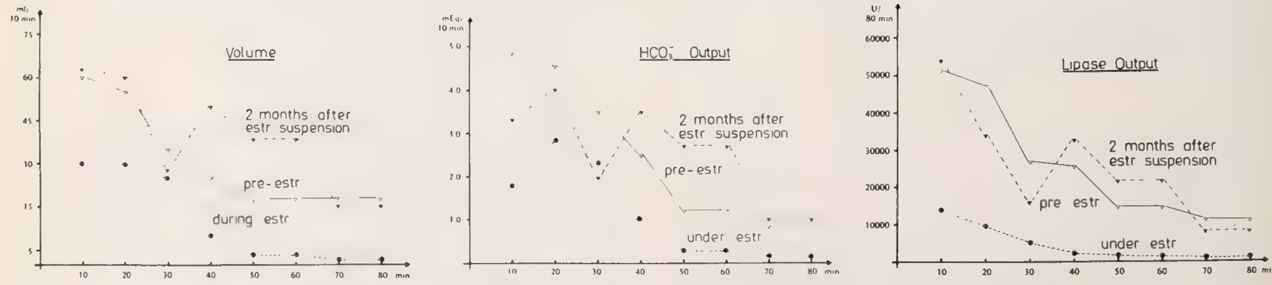

Fig. 5. Sequential changes in postsecretin secretory pattern (volume and bicarbonate and lipase output) in a healthy menopausal woman, before estradiol treatment, during 4-month treatment, and 2 months after it was suspended.

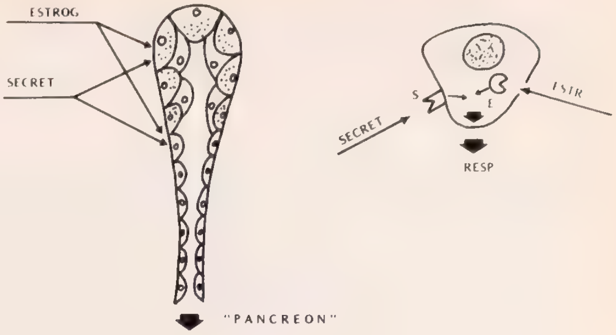

Fig. 6. Hypothesis of interplay between secretin (secret.) and estrogenic (estr.) receptors in "pancreon" cell.

cantly less pancreatic hydrelatic and ecbolic responses to secretin in women over the age of 45 than in men of the same age group (3). This pattern of secretion was related to the fall in plasma estradiol levels observed in menopausal women. It is presumed that normally, estrogen receptors on pancreatic acinar and ductular cells facilitate and/or potentiate secretin receptors of the cell membrane. Hence, the loss of "estrogenic tone" in women at menopause would elicit decreased responsiveness to secretin (Fig. 6).

The initial decrease in secretin response noted in short-term administration of estradiol to menopausal women is in accord with results obtained by injection of estrogen in the rat (7). Thus, increase in intrapancreatic estrogenic tone would augment secretin response, while decrease in estrogenic tone would decrease the pancreatic hydrelatic and ecbolic responses to secretin.

An alternate mechanism whereby plasma estrogen levels might affect pancreatic secretion is an indirect process—by activation of the B cells of the islets of Langerhans. The insulin released would potentiate acetylcholine- and CCK-PZ-induced pancreatic secretion (14). We have shown (7) and others have reported (15, 16) that estradiol administration to rodents results in microscopic and ultrastructural changes in the pancreon and islets, that is, marked hypertrophy of B cells in the islets and depletion of zymogen granules of acinar cells.

The inhibitory phase noted after prolonged administration of estradiol could result from a loss of sensitivity of receptors, a phenomenon observed as a consequence of excessive agonist concentration in contact with cell receptors (17); that this is a reversible phenomenon is witnessed by the rebound noticed in a patient following discontinuance of estrogen administration. Finally, the decreased bile-pancreatic secretory response following prolonged estradiol administration may have a hepatic as well as a pancreatic component; that is, increased hepatic estrogen levels diminish the secretion of the bile-acid-independent fraction of hepatic flow.

The present results may at least partially illuminate the physiopathologic mechanism underlying the postsecretin hydrelatic hypersecretion observed in patients who have one of a group of diseases of the liver and pancreas (19)—biliary cirrhosis, postnecrotic cirrhosis, alcoholic cirrhosis, Zollinger-Ellison syndrome, and hemachromatosis. In these disorders, the enhanced response to secretin has been postulated to result from hypertrophy and hyperplasia of the hepatopancreatic ductular mass as a consequence of parenchymal regeneration and repair following repeated minimal injury. Moreover, in all the disorders in this group, with the exception of Zollinger-Ellison syndrome, relative hyperestrogenism develops gradually as a result of peripheral conversion of plasma testosterone and androstenedione to estrone (20). Even ethanol may play a role in the establishment of an estrogenic tone in the liver and pancreas. Alcohol has been reported to increase the number of estrogen receptors in the liver and, perhaps, the pancreas (21). Indeed, the pancreatic hydrelatic hypersecretion in the cirrhoses has been postulated by Renner et al (22) to be a possible mechanism preventing alcohol-induced pancreatitis by dilution of the ecbolic responses and, thus, inhibiting the precipitation of intraductal protein plugs.

Finally, the decreased pancreatic flow in response to secretin may explain the observed increased incidence of what is termed idiopathic chronic pancreatitis in elderly women. We have previously reported (3) observing in women over the age of 45 hydrelatic depression (lower flow and bicarbonate) coupled with higher lipase output than in younger women. This pattern of secretion was correlated with decreased plasma estrogen levels and its resultant decrease in potentiation of secretin receptors. Such a pattern of secretion would, of course, favor the precipitation of protein plugs in the intraductal system, as has been described by Sarles et al (23–26) for chronic alcoholic pancreatitis.

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Effect of Pyloric Transection on Gastric and Pancreatic Secretion

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Abstract

The effect of pyloric transection on gastric and pancreatic secretion was studied in a group of dogs with chronic esophageal, gastric, and pancreatic fistulas. Pyloric transection did not alter the basal or the sham-feeding, secretin, CCK-PZ, and histamine-stimulated gastric and pancreatic secretions. This indicated that no gastric or pancreatic secretory fibers travel through the pylorus. However, a 30%–50% decrease in serum pancreatic polypeptide (PP) and a onefold increase in serum gastrin were observed after this procedure. The decreased polypeptide level may indicate that at least part of the polypeptide cell secretory fibers pass through the pylorus to the duodenum and then innervate the pancreatic head and uncinate process. The increased serum gastrin may be due to bile reflux alkalinization of the antrum after pyloroplasty.

Previous investigations (1–10) demonstrated that gastric and pancreatic secretions induced by vagal stimulation are mediated by vagal efferents to the gastric and pancreatic glands and perhaps also by vagally released gastrin. A recent study (11) showed that vagally released gastrin may be important for the gastric response to sham feeding but does not contribute to the pancreatic response.

The question arises as to what the major vagal pathway to the pancreas is. Do vagal fibers pass from the stomach to the duodenum and then to the pancreas, or do they travel via extragastric branches?

This study was designed to determine the vagal pathway mediating the gastric and pancreatic response to physiologic vagal stimulation.

Materials and Methods

Three adult mongrel dogs weighing 17–20 kg were prepared with Thomas cannulas placed in the duodenum opposite the major pancreatic duct after preliminary ligation of the accessory duct. A gastric cannula was positioned in the dependent part of the stomach to divert gastric secretion from the duodenum during subsequent experiments as well as to collect gastric juice. The cervical esophagus was tacked to the subcutaneous level, and an esophageal fistula was created about 10 days later. A three-week period was allowed for recovery from these procedures before experiments were started. A 0.9% sodium chloride infusion was maintained throughout each experiment to replace the fluid losses.

Two preliminary basal gastric and pancreatic 15-min collections as well as accompanying blood samples were collected. Then vagal stimulation was induced by a 15-min sham feeding with 500 g of canned dog food. The gastric and pancreatic secretions were collected every 15 min for 2.5 hr after sham feeding, and blood samples were withdrawn and mixed with Trasylol (400 μ/ml of blood). The plasma was separated and frozen at −20°C until radioimmunoassay for gastrin and pancreatic polypeptide was performed. Gastric and pancreatic responses to secretin (Kabi, 4 units/kg/hr), CCK-PZ (Kabi, 4 units/kg/hr), and histamine (0.18 mg/kg/hr) stimulations were collected every 15 min for 1.5 hr after stimulation.

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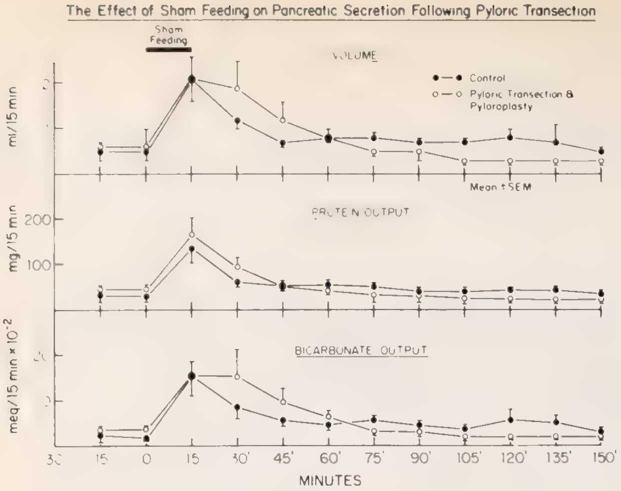

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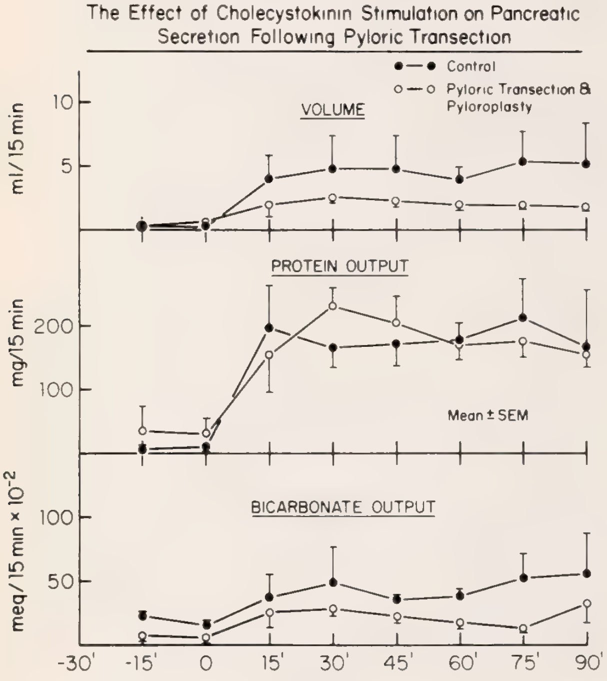

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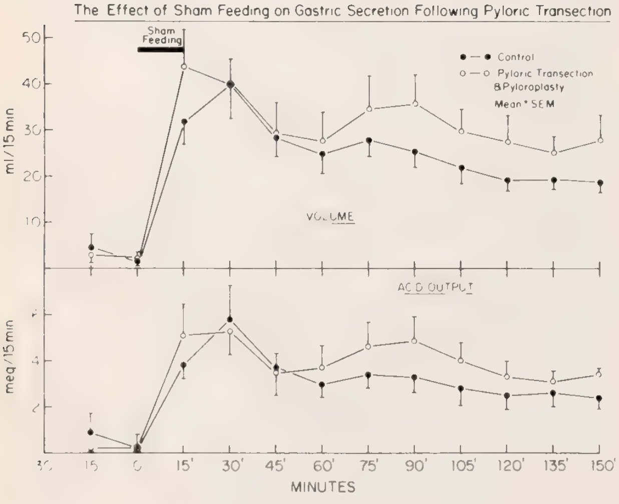

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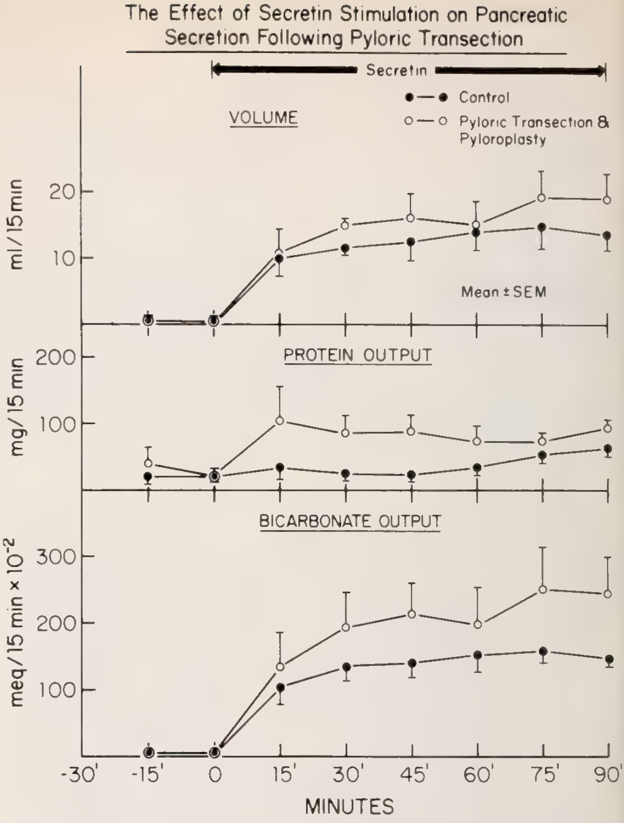

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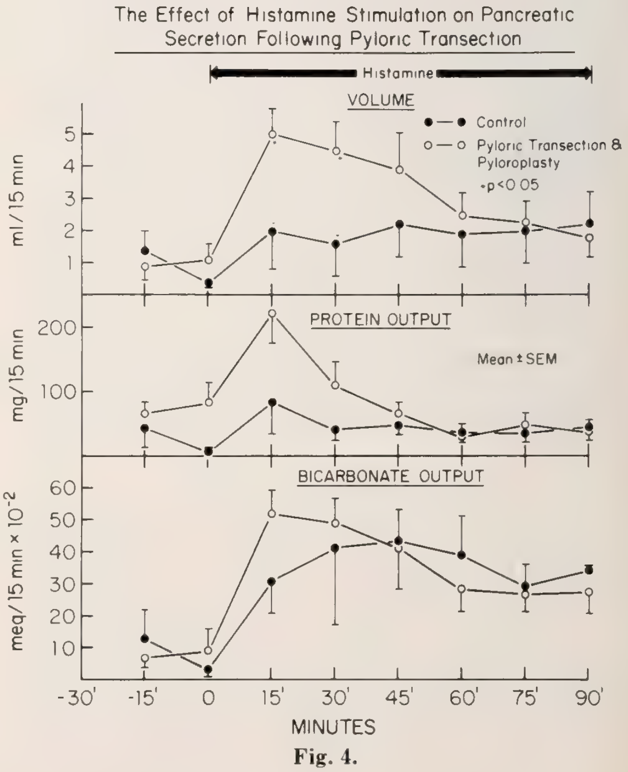

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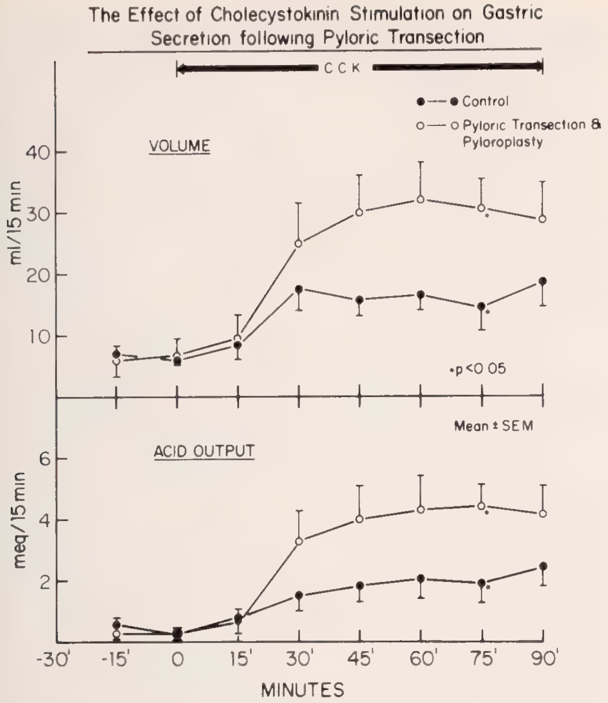

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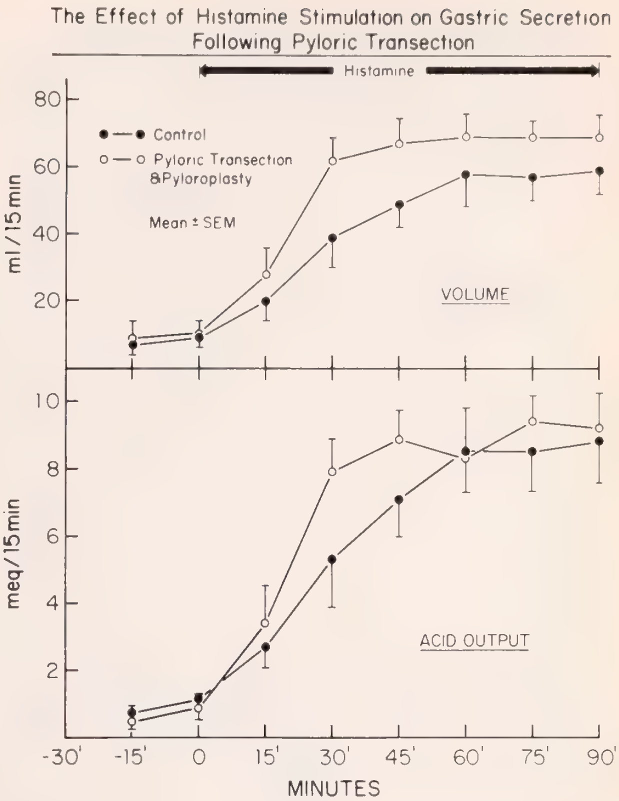

Fig. 7.

Following control studies with sham feeding, secretin, CCK-PZ, and histamine stimulation, all animals were reoperated upon. The pylorus was transected and a Finney pyloroplasty was created. After a three-week recovery period, the previous studies were repeated.

Each sample of pancreatic fluid was analyzed individually for volume (ml/15 min), protein (mg/15 min), and bicarbonate output (mEq/15 min). The protein served as a measurement of enzyme secretion. Protein concentration was determined by Gilford spectrophotometer at 280-nm wavelength, and bicarbonate was determined by Technicon autoanalyzer-II. Gastric specimens were analyzed for volume (ml/15 min) and acid output (mEq/15 min). The acid concentration was measured with automatic titrator (Radiometer Copenhagen). Serum gastrin and pancreatic polypeptide were determined by radioimmunoassay. The postpyloroplasty studies were performed on three separate occasions so that six results were available for each stimulus for comparison. Statistical analysis was done by Student's *t* test. Differences were regarded as significant if $p < 0.05$.

Results

Pancreatic Secretion. Pyloric transection did not alter basal pancreatic secretion or secretion induced by sham-feeding, secretin, CCK-PZ, and histamine (Figs. 1–4). However, transient increase of flow was observed after histamine stimulation ($p < 0.05$) (Fig. 4).

Gastric Secretion. Stimulation of gastric secretion was observed after sham feeding, and this effect was not changed after transection of the pylorus (Fig. 5). Increased gastric secretion was also noticed after CCK-PZ and histamine stimulation (Figs. 6 and 7), and an inhibitory effect was demonstrated following secretin stimulation (Fig. 8). These effects were not altered by pyloric transection.

Serum Gastrin and Pancreatic Polypeptide. Sham feeding resulted in a 30%–50% decrease of serum pancreatic polypeptide (PP) in dogs following pyloric transection, an inhibition that persisted for the duration of observation. Statistical significance was noted in only one experiment (Fig. 9). On the contrary, a doubling of serum gastrin levels was observed in the transected group after sham feeding, an increase that was statistically significant ($p < 0.05$) (Fig. 9).

Discussion

The exact course of vagal secretory fibers to the pancreas is still obscure. It has been shown that the majority of these fibers travel in the celiac division of the dorsal vagal trunk and in the hepatic division of the ventral vagal trunk (12).

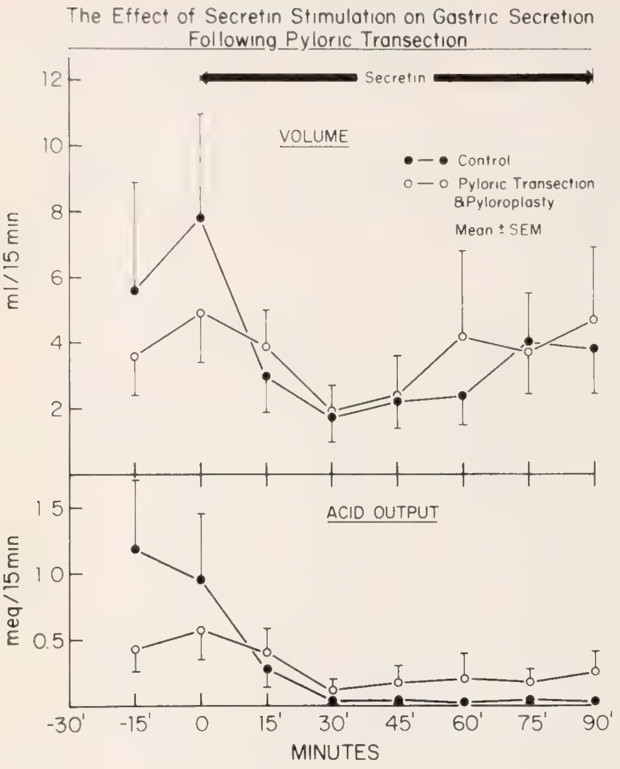

Fig. 8.

Whether fibers from the gastric branches of the vagi also innervate the pancreas is unknown.

Earlier studies have indicated that truncal vagotomy decreases basal pancreatic secretion (13, 14) and decreases the pancreatic response to intestinally perfused stimulants of pancreatic protein secretion (15). Debas et al (6) demonstrated that these effects occur with extragastric vagotomy alone and are caused either by removal of tonic vagal activity directly to the pancreas by interruption of tonic vagal release of hormones from the intestine (6, 15–17), or by removal of the path for vagovagal reflexes from the intestine to the pancreas (6).

With respect to the response to a meal, several reports agree that enzyme secretion is depressed by vagotomy (18, 19). In the present study, transection of the pylorus did not alter the basal and sham-feeding-induced pancreatic secretion. Thus, there appear to be no exocrine secretory fibers traversing the pylorus to reach the pancreas.

Exogenous hormone in the vagotomized animal has been reported to cause either little or no change in pancreatic electrolyte secretion (6, 15, 16, 20) and/or decrease in enzyme response (14, 21). On the other hand, Thambugala and Baron (19) found an increase in protein response to CCK-PZ after truncal vagotomy, and Moreland and Johnson (22) found an increased bicarbonate response to secretin after truncal vagotomy. Our study demonstrated that pyloric transection did not alter secretin, CCK-PZ, or histamine-stimulated pancreatic secretion except for transient volume increases observed following histamine.

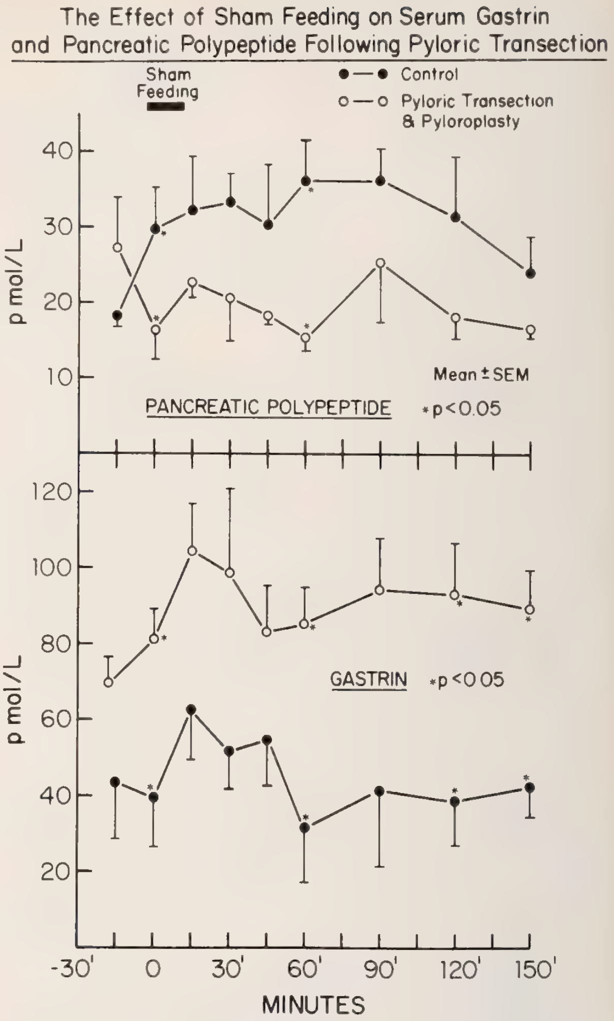

Fig. 9.

Under fasting conditions, the plasma level of PP in dogs and humans ranges from 10 to 50 pmoles/liter (23–25), and in healthy subjects plasma concentration of PP increases significantly with age (24). PP release depends on food stimulation, gastrointestinal hormones, and activity of the autonomic nervous system. Food is the natural and one of the most effective stimulants of PP release (23–27), magnitude of response depending on quantity ingested and foodstuff (24–26). Many reports define the effect of gastrointestinal hormones on the release of PP. Most studies show that exogenous gastrin has minimal effect (26, 28, 31). Endogenous release of gastrin by alkalinization of the stomach also does not significantly alter the PP level in the plasma (28). The secretion of PP was not affected by purified secretin (29), synthetic CCK-PZ (30), or endogenous secretin (26). GIP and VIP are reported to cause secretion of PP from the isolated pancreas (31), whereas somatostatin definitely decreases secretion of PP in pig, dog, and human (24, 29, 31–33). However, bombesin and cerulein have potent action on the release of PP (31, 34). Finally, the stimulatory effect of insulin and other hypoglycemic agents and the inhibitory effect of hyperglycemia appear to be mediated via a vagal reflex. Increase in plasma PP levels following sham feeding in the dog was first observed by Lin and Chance (35). Taylor et al (26) also found a similar increase of human PP concentration after modified sham feeding in man. Schwartz et al (28) sham-fed patients with gastrostomy and found that the mean human PP level was raised by about 47 pmoles/liter in 30 min. Thus, in comparison with the effect of intragastric food, the effect of sham feeding is relatively modest but significant. Schwartz et al (28) and Taylor et al (26), moreover, demonstrated that the cephalic phase of PP release in man was abolished in vagotomized patients and by anticholinergics. Another study (36) showed that truncal vagotomy abolished the early or cephalic phase of PP response and diminished the delayed PP response to food. Finally, Taylor et al (37) found that vagotomy did not significantly alter the plasma basal concentration of PP.

PP-producing cells in mammalian tissues are identified by immunohistochemical procedures. Human PP cells are located mainly at the periphery of the islets (30). Some are found scattered in the exocrine parenchyme and even in the epithelium of the ducts (38, 39). In addition to the human pancreas, PP-producing cells were also identified in the pancreata of dog (38, 40, 41) and other species (40, 41). In fact, in most species, the distribution of PP cells in the pancreas of animals is similar to that in man. Generally, PP cells are numerous in the uncinate process adjacent to the duodenum and scarce in the body and tail of the pancreas (39–43). For this reason, the uncinate process contains four to five times as much PP as is found in the tail by immunoassay.

The present study demonstrated that sham feeding does increase PP secretion and that transection of the pylorus decreases the serum PP response to sham feeding 30%–50%. This suggests that at least part of the PP release fibers must travel through the pylorus to the duodenum and then innervate the PP-secretory cells of the pancreatic head and uncinate process.

Gastrin, acetylcholine, and histamine are important physiological regulators of gastric secretion. The effect of sham feeding on gastric secretion had been well demonstrated (44–49). Chemo- and mechanoreceptors located in the tongue and buccal and nasal cavity are stimulated by tasting, smelling, chewing, and swallowing food. Afferent impulses, relayed through the vagal nucleus and vagal efferent fibers to the stomach, stimulate gastric secretion despite diversion of the food from the stomach. The gastric divisions of ventral and dorsal vagi act directly on the parietal cells to stimulate acid secretion (4, 49) as well as on the antral gastrin cells to stimulate gastrin release (4, 49). The mediator at the parietal cell is acetylcholine, and for gastrin release, probably bombesin (50, 51). Inhibition of gastrin release by somatostatin (49, 52) is also vagally mediated.

Augmentation of gastric secretion by sham feeding observed in this study was not affected by pyloric transection, indicating that vagal innervation to the stomach was not affected by this procedure. The significant increase of serum gastrin noted in the basal state as well as after sham feeding after pyloroplasty may result from bile reflux and alkalinization of the antrum, which would stimulate the gastrin release.

Exogenous CCK-PZ as a weak stimulant of gastric secretion (53) and the potent action of histamine on gastric secretion have been well documented since early in this century. The data show that pyloric transection slightly increases CCK-PZ and histamine-stimulated gastric secretion. This effect is no doubt due to synergism between the increased gastrin released and the exogenous CCK-PZ and histamine administered. On the other hand, exogenous secretin was shown to inhibit acid secretion and gastrin release (54). Lee et al (55) demonstrated that elimination of circulating free secretin by a highly specific antisecretin antibody resulted in a significant increase in both gastric acid secretion and plasma gastrin following a meal. They suggested that secretin is an enterogastrone in the dog. The inhibitory effect of secretion was found in the study and was not affected by pyloric transection.

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Is Basal Bile-Pancreatic Secretion Influenced by Gastric Juice Diversion in the Rat?

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Basal bile-pancreatic secretion in the rat is subject to negative feedback by bile and pancreatic juice. As early as 1917, this mechanism was implied by the discovery of a heat-labile trypsin inhibitor that caused poor growth in rats fed on raw soybean meal, which interfered with normal tryptic activity (1). It was subsequently shown that rodents and poultry fed on a diet containing the isolated soybean trypsin inhibitor developed large pancreases, with an increased capacity to secrete pancreatic digestive enzymes (2–10). It was presumed that the poor growth of soybean-fed animals resulted from increased fecal nitrogen loss due to the enhanced pancreatic secretions—a form of protein-losing enteropathy.

Further study revealed that diversion of bile and pancreatic juice from the intestine increases—and return to the duodenum decreases—pancreatic enzyme secretion as well as fluid and bicarbonate secretion (11–21). This phenomena has also been observed in pigs (22, 23). Trypsin has the same effect as pancreatic juice. In dogs, unlike rats and pigs, basal pancreatic secretion is not under negative feedback control by duodenal tryptic activity (24).

The reports in the literature on humans are contradictory. According to Forell et al (25), bile produces a significant increase in all pancreatic secretory parameters by release of cholecystokinin-pancreozymin (CCK-PZ) from the intestinal mucosa. For Osnes and Hanssen (26), secretin plays an important role in the exocrine pancreatic secretion induced by the intraduodenal infusion of bile. Recently it has been shown that, in humans, perfusion of a high concentration of trypsin during continuous infusion of phenylalanine into the duodenum triggers negative feedback signals to pancreatic enzyme secretion (27). Owyang et al (28) reported that administration of atropine completely inhibits flow and osmolarity-stimulated pancreatic enzyme secretion but only partially inhibits phenylalanine-stimulated enzyme secretion. This study also indicated that the intestinal phase of human pancreatic exocrine secretion is under both hormonal and neural control. Intraduodenal trypsin inhibited hormonal-mediated, but not cholinergic-mediated, pancreatic enzyme secretion. In humans, CCK-PZ plasma levels are higher in patients with exocrine pancreatic insufficiency, probably reflecting failure to provide feedback down-modulation of CCK-PZ release (29).

In the rat, according to Laporte and Tremolieres (11, 12), trypsin, after its absorption into blood, exerts a secretory brake on the cells of the "pancreon" (the pancreas secretory unit—ducts as well as acinar tissue). This brake is removed by CCK-PZ. Green and Lyman (13) have hypothesized that removal of trypsin and chymotrypsin activity from the intestine by diversion of pancreatic juice, or by administration of trypsin-inhibitor or dietary proteins permits the release of CCK-PZ in the upper small intestine from I cells. Under these conditions, there is a rise of plasma CCK-PZ (30).

In the pig, secretin, also, appears to be in-

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volved in negative feedback affecting exocrine pancreatic secretion (31), since diversion of pancreatic juice from the duodenum causes an increase in the volume of juice and in the amount of protein secreted, without any significant variation in concentration. These secretory changes are accompanied by a significant rise in the portal and peripheral blood levels of secretin (31).

Chronic diversion of bile-pancreatic juice from the rat duodenum into the terminal ileum produced rapid, marked pancreatic growth and increase in the weight of the small intestine, especially of its distal segment, (32); these changes are not seen following long-term administration of CCK-PZ. Indeed, chronic obstructive jaundice in the rat produced by ligation of the bile duct results in increased plasma levels of CCK-PZ and significant enlargement of the pancreas (33). Finally, combining obstructive jaundice with ingestion of trypsin-inhibitor further increases the weight of the pancreas (34). The protein content of the pancreas remains unaffected. Serum amylase is depressed by this experimental obstructive jaundice, along with hypersecretion of exocrine pancreas, a clinical association observed in humans.

Another major factor contributing to the regulation of bile-pancreatic secretion in rodents is rising intrapancreatic cholinergic tone. This increased cholinergic tone is the result of increased nervous impulses traversing short, medium, and long (vago-vagal) duodenopancreatic reflexes. Their participation after the diversion of bile-pancreatic secretion from the duodenum would be a consequence of the activation of nerve arc reflexes by the paracrine effects of released CCK-PZ in the duodenal wall (35). Simultaneously, there also might be a removal of an inhibitory component that would normally result from activation by trypsin and chymotrypsin of a negative duodenopancreatic reflex. An inhibitory peptide or neurotransmitter (pancreatic polypeptide? somatostatin? serotonin?) could be involved (36–41). This participation of negative and positive duodenopancreatic reflexes is supported by the observation that after administration of propantheline bromide (Pro-Banthine), an intraduodenal infusion of trypsin does not depress the duodenally diverted bile-pancreatic secretion (42). A similar phenomenon is observed in vagotomized animals in which the addition of trypsin inhibitor to the infusate does not alter protein output (42). Also supporting the participation of duodenopancreatic reflexes after diversion of bile-pancreatic secretion in the rat is a change in secretory pattern after chronic truncal vagotomy, namely, a significant increase of flow and bicarbonate output (indicating removal of an inhibitory factor?), as well as a marked delay in reaching the plateau levels of protein secretion following stimulation (indicating lack of firing of positive nervous impulses through long arc reflexes?). Yet total protein secretion in 240 minutes is not significantly different from nonvagotomized animals (unpublished observations). Finally, chronic administration of atropine increases the incremental—that is, above basal—protein and fluid response to intraduodenal infusion of ovomucoid trypsin inhibitor (43). This finding indicates that chronic removal of cholinergic tone in the pancreas leads to increased pancreatic secretory capacity.

That at the plateau of stimulated, diverted bile-pancreatic secretion, without intestinal recirculation, there is a high intrapancreatic cholinergic tone is supported by the secretory depression induced by atropine (44, 45) and the failure of a cholinomimetic (pilocarpine) to increase secretion (45, 46), and by the observation that exogenous CCK-PZ is unable to augment secretion (19, 47).

On the other hand, the addition of an alpha-amylase inhibitor to the diet for three weeks or for more prolonged periods (three weeks to three months) results in no change in pancreatic weight or in DNA. Hence, unlike the situation with proteases, a negative feedback does not exist between alpha-amylase concentrations in the gut and the pancreas (48).

In rats with a copper deficient diet that are fed penicillamine, the result is atrophy of the exocrine pancreas by selective fatty degeneration of the acinar cells. In these rodents, there is a marked increase of plasma CCK-PZ concentration, and a significant decrease of the CCK-PZ concentration in the proximal duodenum, while intestinal GIP concentration and tissue concentration of GIP in the duodenum, jejunum, and gastric-antrum gastrin remain unaltered. These data suggest that the negative feedback control of pancreatic enzyme secretion is mediated by the release of CCK-PZ (49, 50).

In one uncontrolled study, a beneficial effect of a soybean flour diet on pancreatic function has been described in patients with chronic pancreatitis (51). This effect, however, could not be reproduced in a controlled investigation (52).

Brand and Morgan (53) have recently studied the influence of luminal bile salts on pancreatic growth in rats by feeding cholestyramine,

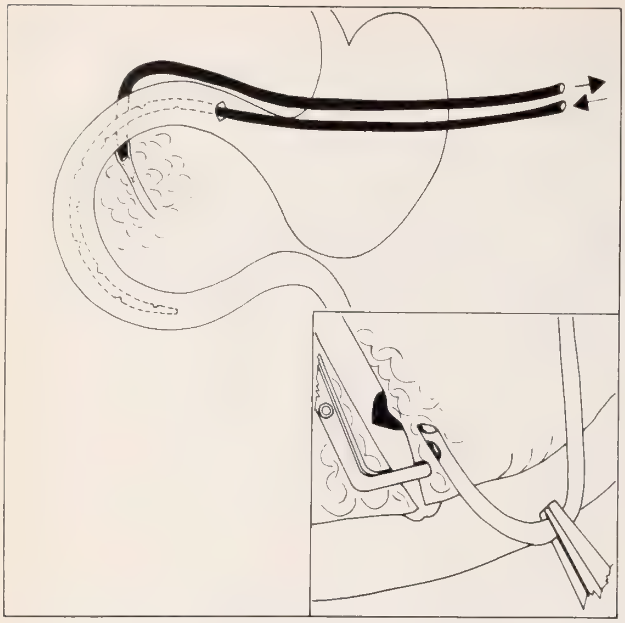


Fig. 1. Biliary-pancreatic fistula.

an anion-exchange resin that binds luminal bile salts, and by chronic bile diversion to the urinary bladder. Cholestyramine stimulated pancreatic growth, increasing both its size and DNA content. A dose of 0.5% was ineffective, but feeding 2%, 6%, and 10% cholestyramine (w/w) gave the same degree of pancreatic enlargement. Feeding cholestyramine increased both the basal pancreatic secretion and the secretory response to exogenous CCK-PZ. Chronic bile diversion to the urinary tract caused pancreatic growth similar to that seen in cholestyramine-fed animals. Brand and Morgan (53) concluded that the pancreatic enlargement and the increase of basal pancreatic secretion is similar in mechanisms to that seen after soybean trypsin-inhibitor.

Goke et al (54) have reported that chronic oral administration of camostate, a specific serine protease inhibitor, phenylacetate methanesulfonate (Foy-305, N,N-dimethyl-carbamoylmethyl-p9p-guanidinobenzoylozy) with a molecular weight of 495.5 daltons, induces pancreas hypertrophy in rats. Applied to the lumen of an isolated perfused rat duodenum, it releases CCK-PZ into the portal vein.

Another facet in the mechanisms that control basal bile pancreatic secretion is duodenal acidity. Noda et al (55) have recently shown evidence that, in the rat, the entry of gastric juice into the duodenum contributes substantially to the pancreatic juice hypersecretion seen following its diversion from the duodenum. This hypersecretion would result from increased duodenal acidity due to the absence of pancreatic bicarbonate or from an increased sensitivity of receptors resulting from the absence of trypsin, or from both these factors. In animals with pyloric ligation, in which gastric juice was excluded from the duodenum, basal pancreatic secretion was small and not increased by bile-juice diversion. The pancreata of these animals were, however, responsive to HCl instilled into the duodenum.

The present experiments were designed to elucidate whether or not gastric juice diversion influences basal bile-pancreatic secretion at 24 hours and 48 hours after creation of a biliary pancreatic fistula.

Material and Methods

Experiments were carried out in male Wistar rats. Four groups of animals were studied: two without and two with a gastric cannula inserted in the lowest part of the rumen and brought to the exterior through a paramedian incision in the upper left quadrant of the abdominal wall. The animals with and without gastric cannula were paired by weight. There were no statistical differences among the body weight mean values which were the following: 408 ± 20.71, 409 ± 23.47, 390 ± 7.17, 380 ± 9.93 gm. The first and second pair of animals were studied at 24 hours and 48 hours after surgery.

Biliary-Pancreatic Fistula. Biliary-pancreatic fistulas were fashioned in all four series of rats (Fig. 1). After entering the abdomen through a midline incision and under the dissecting microscope, a plastic catheter (outer diameter 0.65) was inserted in the terminal end of the bile duct. The distal segment of the duct was ligated with 6-0 silk and the catheter secured with another ligature of the same material. The catheter was given a hook form, and the tip of the short segment of the hook was bevelled to facilitate its entrance into the bile duct. Close to this tip, a small hole was made in the catheter opposite to the bevelled side. This was done to prevent interruption of the bile-pancreatic flow if movement of the plastic catheter caused its tip to press against the hepatic duct wall. The long segment of the hook was brought to the subcutaneous tissue at the left side of the midline laparatomy. Its course was from right to left, crossing the abdominal cavity in a horizontal direction under the liver and in front of the anterior surface of the stomach. Prolonged by a Silastic catheter, it was then brought to the neck through a subcutaneous tunnel. Another Silastic catheter, 8 cm long, with several holes placed alternatively around its circumference, was inserted through a small incision in the posterior wall of the duodenum close to the pylorus. At this level, it was secured by a purse-string suture. Prolonged by another plastic catheter (outer diameter 0.65) and after following a right to left course, it was brought to the subcutaneous level through the incision previously performed at the left side of the midline laparatomy. From here, through the subcutaneous tunnel, it was transferred to the neck. Both catheters in the neck were connected to each other to allow recirculation of bile-pancreatic juice into the gut. Marked elasticity of the Silastic tube within the duodenum prevented deformation of the duodenal *C* form.

This approach has two fundamental advantages. First, it permits unfolding of the mesoduodenum pancreas through which the bile duct courses. This permanent stretching of the mesoduodenum by the intraduodenal silastic tubing prevents its shrinkage and retraction and, thus, avoids impairment of bile flow and pancreatic drainage through the several small ducts that enter the distal segment of the hepatic duct. Second, both the Silastic intraduodenal tube inserted through the posterior wall of the first part of the duodenum and the hook of the plastic catheter draining the bile duct allow normal positioning of the duodenum, a prerequisite to maintaining an unimpaired flow of the bile-pancreatic juice to the neck and its recirculation into the upper small bowel.

Gastric Fistula. Gastric fistulas were made in two series of rats. A stainless steel cannula, as described by Petersen and Grossman (19), was inserted in the lowest part of the rumen, secured by a purse-string suture, and led out through a paramedian incision in the upper left of the abdominal wall.

Surgical procedures were always performed during the morning hours; the animals were not previously deprived of food. Between tests, animals were kept in individual cages. Rats were tested during the first two days following surgery. During the first 48 hours, the animals were allowed to drink tap water but food was withheld. After 48 hours, when the test of this day had been completed, the rats were killed and autopsied.

Secretory Studies. All testing was performed during the morning hours, between 8 AM and 11 AM, at room temperature (25–27°C). Rats were taken from their individual cages and placed in a Bollman restraining cage. The tubes in the neck were disconnected and the bile-pancreatic juice was collected in small calibrated tubes immersed in crushed ice. The collection periods were 30 min each for 180 min. In the rats with gastric fistula, the fistulas were open during the 20-minute collection of bile-pancreatic juice. The gastric juice was permitted to drip into a small calibrated tube immersed in crushed ice.

The following parameters and analytic methods were chosen for study:

Basal Bile-Pancreatic Juice: (a) Volume (ml/30 min) was measured with a precision of 0.1 ml; (b) bicarbonate concentration (HCO_3^- in mEq/l) was determined by the titrimetric method of Lehmann et al (56); (c) HCO_3^- output was calculated and was expressed as μEq/30 min; (d) protein concentration was measured according to the method described by Gornall et al (57); (e) protein output was expressed as mg/30 min.

Basal Gastric Juice: (a) Volume (ml/30 min); (b) hydrochloric acid concentration (HCl in mEq/l) was estimated by titration to pH 7.0 with 0.01 N sodium hydroxide; (c) HCl output was calculated and expressed as μEq/30 min.

Statistical Analysis: The mean and standard error of the results were calculated. The significance between means was evaluated using Student's *t* test (58). Values less than 5% ($p < 0.05$) were interpreted as significant.

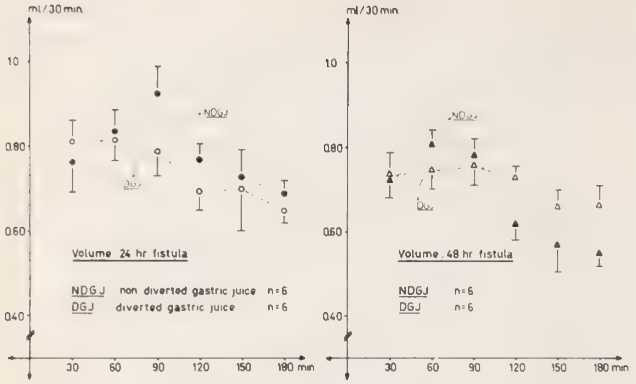
Results

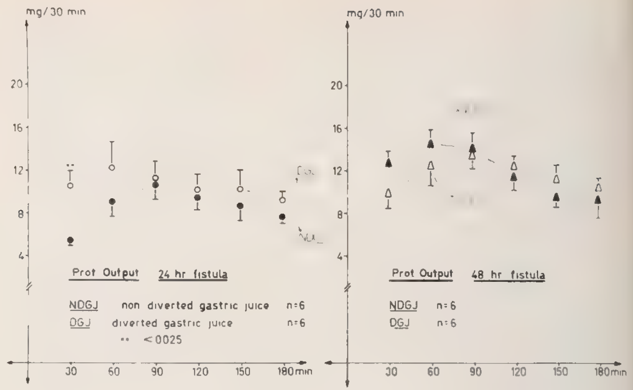
Basal Bile-Pancreatic Juice. At 24 hours after the fistula operation, basal pancreatic flows were similar in rats with and rats without diversion of gastric juice (Fig. 2). Bicarbonate concentration (Fig. 3) and bicarbonate output (Fig. 4) were significantly higher in animals with nondiverted gastric secretion than in those with gastric juice diverted. Tests for protein concentration (Fig. 5) and output (Fig. 6) yielded values that were similar in the animals with and animals without gastric cannula.

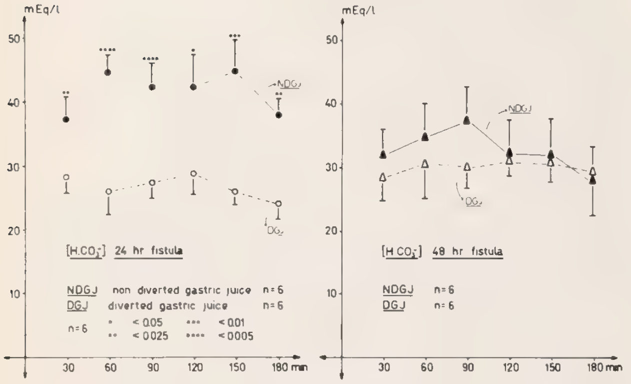
At 48 hours postoperative, none of the parameters of basal-bile pancreatic juice—measures of volume, bicarbonate concentration and output, protein concentration and output—showed statistically significant differences (Figs. 2–6).

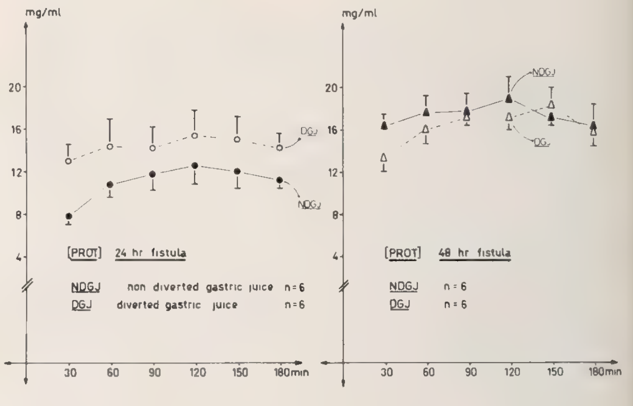
Basal Gastric Juice. At 24 hours after the fistula operation, the HCl output, in the first 60 minutes, averaged 45.16 ± 15.11 μEq. In the second hour, HCl output decreased to 10.51 ± 3.20 μEq. At 48 hours after surgery, HCl output, in the first hour, averaged 13.23 ± 3.62 μEq. This decreased to 3.55 ± 1.06 in the second 60 minutes.

Statistical analysis showed that the differences between means of the first 60 minutes at 24 hours and 48 hours after the operation were significant at the $p < 0.05$ level.









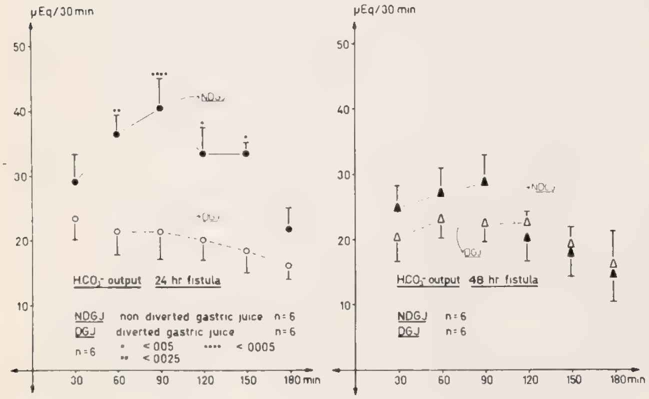


Fig. 2. Top left Volume of basal bile-pancreatic secretion at 24 hours postcreation of fistula in tests with nondiverted gastric juice (NDGJ) and with diverted gastric juice (DGJ). **Right** Volume at 48 hours post fistula surgery. Key as above.

Fig. 3. Center left Bicarbonate concentration at 24 hours after creation of fistula, with gastric juice diverted (DGJ) and not diverted (NDGJ). **Right** Bicarbonate concentration at 48 hours.

Fig. 4. Bottom left Bicarbonate output (μEq/30 minutes) at 24 hours after creation of fistula, with gastric juice diverted (DGJ) and not diverted (NDGJ). **Right** Bicarbonate output (μEq/30 minutes) at 48 hours.

Fig. 5. Top right Protein concentration (mg/ml) at 24 hours after creation of fistula, with gastric juice diverted (DGJ) and not diverted (NDGJ). **Right** Protein concentration (mg/ml) at 48 hours.

Fig. 6. Above left Protein output (mg/30 min) at 24 hours after the creation of the fistula, with gastric juice diverted (GJD) and not diverted (NGJD). **Right** Protein output (mg/30 min) at 48 hours.

Discussion

The aim of the experiments was to ascertain whether diversion of gastric juice altered the absolute values and/or the secretory pattern of basal bile-pancreatic secretion. Analysis of the results with and without gastric cannula shows that, in general, the absolute values and the secretory pattern of the basal bile-pancreatic secretion parameters are identical (Figs. 2–6).

Bicarbonate concentration and output (Figs. 3, 4) are the exceptions, being, at 24 hours, significantly higher in tests of rats with nondiverted gastric juice than in tests of those with an open gastric cannula. However, these differences are effaced at 48 hours.

The latter results are in contradistinction to those reported by Noda et al (55). Indeed, these authors found, in an animal model with chronic external pancreatic and gastric fistulas and choledocho-enterostomy, that pancreatic secretion of water and protein after juice diversion is significantly lower when the gastric fistula is open than when closed. They concluded that the initial hypersecretion of pancreatic juice and protein following its diversion from the duodenum is a consequence of the entry of gastric juice into the intestine.

The basis of the differences between the present tests and those reported by Noda et al (55) may be explained by the protocol in that, after operation, their rodents were placed in Bollman restraining cages, where they remained until the experiments were completed. Studies were performed from the fourth to the seventh day after surgery. This protocol is unphysiologic, since the basal bile-pancreatic secretory flow rate after the 48-hour postoperative period becomes irregular and unreliable; due presumably to an inflammatory infiltration of the pancreatic gland. This is frequently found in postmortem examination of animals sacrificed three or more days after fistula formation, and is most probably due to an infective process facilitated by the presence of the intracholedochal catheter. Another unphysiologic aspect of the Noda protocol is the ligation of the bile duct at the hilus of the liver in order to isolate bile from pancreatic juice. This creates two abnormal situations. First, it prevents the “suction-pump” effect exerted normally by the flow of bile on the drainage of pancreatic juice through several small pancreatic ducts that enter the bile duct. Second, the suture around the proximal common bile duct may injure nearby nerve fibers and, thus, depress spontaneous pancreatic secretion. This phenomenon was reported by Geratz and Lamb (59) but disregarded by Staub and Sarles (17), who related the depression to observations of anesthetized rats. Staub and Sarles insist that in conscious rats, chronic obstruction of the common bile duct increases basal pancreatic secretion. This concept is supported by the recent findings of Ami et al (60), who reported that, in the rat, obstructive jaundice for 7 days induces trophic changes in the duodenum and pancreas, associated with elevated plasma levels of secretin and gastrin, as well as with enhanced levels of duodenal CCK-PZ.

Another striking difference between the results reported by Noda et al (55) and the present ones are the rates of gastric acid secretion. Indeed, in their studies, the basal gastric acid secretion was 100–130 μEq/30 min. In the present experiments, in tests after the fistula was in place for 24 hours, the rate of gastric acid secretion was 45 μEq/60 min. This level had decreased significantly by 48 hours to 13 μEq/60 min. These differences are presumed to derive from the times of study, as well as procedural differences with respect to restraint of the animals in the intervals between study. It is tempting to assume that restraint in a Bollman cage for four to seven days, as in the Noda experiments, would induce an increase of sympathoadrenal tone. But sympathomimetics have been reported to reduce mucosal blood flow and depress gastric acid secretion (61).

The significant decrease in gastric secretory rate at 48 hours after surgery, compared to readings at 24 hours, requires elucidation. Borella and Herr (62) reported that, in acute experiments, the basal gastric acid secretion in unanesthetized rats without pyloric ligation was twice as high as in rats with chronic fistula preparations. They postulated that the high rate of acid secretion in the acute-fistula rat might be due to the liberation of secretagogues from traumatized tissues. They also stressed that in rats with gastric fistulas, only half of the acid produced is, in fact, recovered. This would imply that when the gastric cannula is open, as in the present studies, gastric juice continues to enter the duodenum. This might be a significant factor in bile-pancreatic hypersecretion following the diversion of gastric secretion from the duodenum. This concept, suggested by Noda et al (55), is not supported by data in the literature. Petersen and Grossman (19) have reported stimulation of bile-pancreatic secretion triggered by diversion of gastric secretion without intestinal recirculation. Furthermore, CCK-PZ is released into the circulation from the upper intestinal mucosa after diversion of either bile or pancreatic juice (29, 30). Finally, under the latter conditions, there is an increase in the intrapancreatic cholinergic tone triggered, on the one hand, by activation of positive duodeno-pancreatic reflexes and, on the other, by concomitant removal of a negative component. That during the plateau stage of bile-pancreatic secretion (average of the 2nd and 3rd period) there is cholinergic activity is proved by the fact that blocking muscarinic receptors (atropine) induces a depression of all the secretory parameters (15, 16, 43–45). The same result is seen with xylocaine instillations into the gastrointestinal tract: a decrease in basal bile-pancreatic secretion by interruption of the afferent limb of the duodenopancreatic reflex system (63). Louie et al (64), investigating the mechanisms responsible for the release of CCK-PZ following diversion of bile-pancreatic juices in rats, demonstrated that intraduodenal perfusion of lidocaine, infusion of tetrodotoxin into the superior mesenteric artery, and intravenous atropine each inhibited the re-

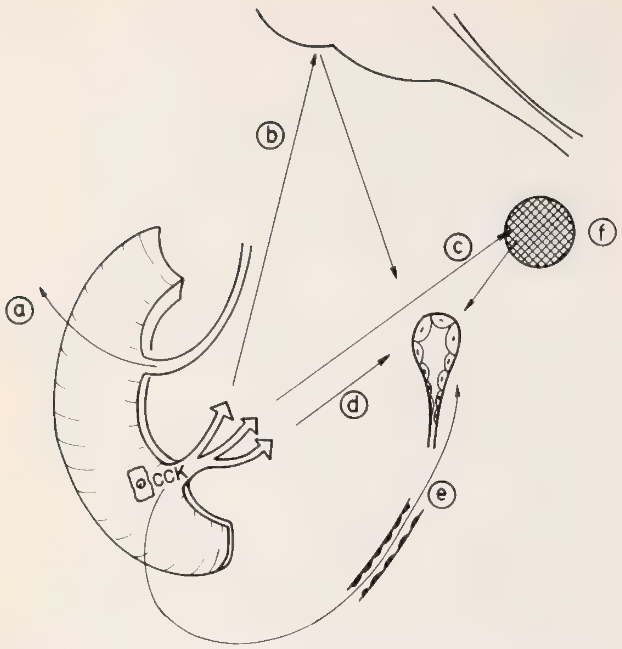


Fig. 7. Changes in basal bile-pancreatic secretions induced by diversion without intestinal recirculation (a). CCK is released from I cells in duodenal mucosa and exerts its effects on "pancreon" units through blood (e) and by triggering paracrinically duodenopancreatic reflexes by means of short (d), medium (c), integrated in celiac plexus (f), and long arc reflexes (b). Simultaneous with this positive cholinergic reflex would be a lessening influence of negative cholinergic reflexes (PP?).

lease of CCK-PZ, indicating that secretion of this peptide is mediated by a cholinergic pathway.

The present experiments indicate a striking discrepancy between the hydrelatic and ecbolic data on changes in basal bile-pancreatic secretion seen after the diversion, or nondiversion, of gastric juice for 24 hours (Figs. 2–6). Indeed, the increase in bicarbonate concentration and output in studies relating to nondiverted gastric juice is not accompanied by augmentation of protein secretion. Accepting that HCl entry into the duodenum is a pivotal factor, it is logical to infer that only hormones of the secretin family have been released from the mucosa of the upper intestine. Another possible explanation for the failure of increase in the ecbolic component of bile-pancreatic secretion in the studies in which gastric juice was not diverted, compared to those in which it was diverted, is that ecobolic secretion had already reached its highest level after the diversion of bile-gastric secretion studies because either CCK-PZ had already been maximally released from the intestinal mucosa (19) and/or the acinar cells of the pancreon units were at their highest secretory capacity, as a result of the combined effects of released CCK and firing of duodenopancreatic reflexes (Fig. 7). A final possible factor explaining the difference observed in secretion in the tests with nondiverted and diverted gastric juice, a difference limited to the hydrelatic component measured 24 hours after the creation of the fistula, is a significantly higher gastric acid juice load entering the duodenum at 24 hours than at 48 hours.

Summary

The effect of diversion of gastric juice on basal bile-pancreatic secretion was analyzed in four series of adult male Wistar rats. Animals with and others without gastric cannula were studied at 24 hours and 48 hours after production of a biliary-pancreatic fistula. Gastric acid secretion after 24 hours was significantly higher than at 48 hours.

Without intestinal recirculation of bile-pancreatic juice, the basal hydrelatic and ecbolic parameters of bile-pancreatic secretion, studied over 180 minutes, showed a pattern of progressively increasing values up to the third 30-minute period, followed by a progressive decline to the end of investigation. Similar data was observed at 24 and 48 hours following surgery, as well as at 48 hours, whether or not gastric juice was diverted.

At 24 hours, with gastric juice diversion, there were no changes in flow or protein output, compared to findings in rodents with gastric secretory diversion. However, bicarbonate concentration and bicarbonate output were significantly higher in the rats with nondiverted gastric juice than in the series with gastric-juice diversion.

These findings indicate that an acid load in the duodenum is unable to alter the protein component of bile-pancreatic secretion observed after its diversion from the duodenum without intestinal recirculation. The data suggests that the pancreon units are secreting ecbolically at a maximum level and/or that ecbolic influences (CCK-PZ, duodenopancreatic circuits) are at their highest activity. The changes in bicarbonate concentration observed at 24 hours are interpreted to result from the increased acid load at 24 hours compared to 48 hours.

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Partial Pancreatectomy Augments Pancreatic Cancer in the Rat

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Abstract

Rats subjected to partial pancreatectomy were compared with control animals in their response to injections of a genotoxic pancreatic carcinogen (azaserine) and to the effects of a diet of raw soya flour. Partial pancreatectomy augmented the carcinogenic effects of azaserine, but did not influence the carcinogenic effects of the diet. We conclude that partial pancreatectomy enhances the effect of a genotoxic carcinogen, just as partial hepatectomy promotes hepatic carcinogenesis.

We have previously shown that proliferation of pancreatic acinar cells elicited by a raw soya-flour-containing diet (1) or by DL-ethionine-induced pancreatitis (2) potentiates the carcinogenic effects of a weak pancreatic carcinogen (azaserine). In the present study, we have investigated the effects of pancreatic resection, which provides a different type of stimulus to pancreatic growth, on pancreatic carcinogenesis.

Methods

A total of 163 male Wistar rats, weighing 400–450 gm at the start of the study, were divided into two groups, one of which contained rats subjected to pancreatectomy, while the remaining animals served as controls.

All pancreatectomies were performed using pentobarbitone sodium as anesthetic (Sagatal, May & Baker Ltd., Dagenham, England). Resection involved 50%–75% of the gland. The technique of pancreatectomy has been described previously (3).

Postoperatively, each group was further subdivided into three subgroups (Table), each subgroup receiving one of three treatment schedules, all begun 3 days postoperatively. Azaserine (Calbiochem, Bishops Stortford, England) was administered intraperitoneally in a dose of 5 mg/kg body weight in 0.2 ml of 0.15 mol/l sodium chloride, once weekly for up to 85 weeks. The rats were fed a non-soya-containing diet. A second subgroup received 0.2 ml saline intraperitoneally once weekly and the same non-soya-containing diet. The third subgroup was fed a diet of raw soya flour for as long as two years. The composition of the diets has been described previously (4).

At the end of the second year of study, all the rats had been killed. The pancreases and other organs were removed, examined, and weighed. All tissues were fixed in buffered formaldehyde and prepared for routine histological examination. Twelve blocks were taken from each pancreas. Sections were stained with hematoxylin and eosin. The sections were examined microscopically and volume densities of the pancreatic nodules were measured according to the method of Weibel (5), as described previously (1).

Results

Eleven rats died or had to be killed during the immediate postoperative period because they were suffering from either acute pancreatitis or obstructive jaundice.

Body Weights. All animals lost body weight during the first postoperative week, but then rap-

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TABLE

Effects of Treatment Regimens on Rat Pancreas

Group	Treatment	No. of rats	Age (weeks)	Microscopic nodules	Macroscopic nodules	Adenomatous nodules	Volume densities of micr. nodules	% Carcinomas
1	RSF	43	>60	98	98	84	78	12
2	NSC + S	27	>60	0	0	0	0	0
3	NSC + A	15	>60	93	87	40	53	0
4	P + RSF	2	31–60	0	0	0	0	0
		14	>60	100	100	85	78	7
5	P + NSC + S	2	<30	0	0	0	0	0
		1	31–60	0	0	0	0	0
		19	>60	16	5	0	0	0
6	P + NSC + A	1	<30	0	0	0	0	0
		1	31–60	100	100	0	0	0
		27	>60	100	100	63	64	22

P, pancreatectomy; NSC, non-soya-containing diet; RSF, raw soya flour diet; A, azaserine injections; S, saline injections. Values for nodules, volume densities, and carcinomas represent percentages of animals and pancreases. Volume density values indicate medians.

idly recovered, so that their weight did not differ from the control group receiving the same regimen. The rats receiving raw soya flour were lighter than rats given the nonsoya diet.

Pancreatic Weights. All saline-injected rats had pancreases of similar size, irrespective of whether or not the animal had undergone pancreatic resection at the beginning of the study (groups 2 and 5, Table and Fig.). The pancreases in the remaining groups were significantly larger and similar, irrespective of operative or dietary schedule (groups 1, 3, 4, and 6, Table and Fig.).

Other Neoplasms. Two of the pancreatectomized and two of the unoperated-on rats given azaserine developed adenocarcinoma of the kidney. One unoperated-on animal given azaserine developed a fibrosarcoma of the kidney. One pancreatectomized rat which had been injected with saline developed an intraabdominal spindle-cell carcinoma.

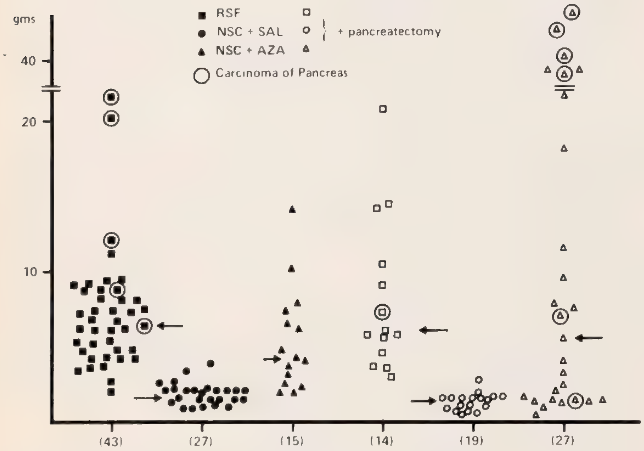

Fig. Pancreatic weights of rats in different treatment groups. Each point represents weight of pancreas of one rat. Numbers in parentheses indicate number of rats in each group. Horizontal bars with arrows indicate median values. RSF, raw soya flour (closed and open squares); NSC + SAL, non-soya-containing diet plus saline injections (closed and open circles); NSC + AZA, non-soya-containing diet plus azaserine injections (closed and open triangles). Closed symbols, unoperated-on rats; open symbols, pancreatectomized rats. Large circles surrounding symbols = animals which developed pancreatic cancer.

Discussion

It has previously been reported that the rat pancreas regenerates after pancreatectomy (6). This study has confirmed that the pancreatic weights of rats revert to normal after resection of as much as 75% of the pancreas and that pancreatic resection is not associated with long-term changes in either body weight or length of life of the animals. Moreover, pancreatectomy did not interfere with the hyperplastic response of the pancreas to the raw soya flour diet or to azaserine, so that at the end of the study the pancreatic weights of resected and control animals were indistinguishable in all three groups (Fig.).

We have previously shown that pancreatic cellular proliferation provoked either by raw soya-flour-containing diets (7) or by DL-ethionine-induced pancreatitis (2) promotes the development of pancreatic cancer in rats exposed to a genotoxic pancreatic carcinogen (such as azaserine). The present study has shown that pancreatic growth following pancreatectomy also enhances the development of pancreatic cancer. However, pancreatectomy does not further increase the weak pancreatic carcinogenic effects of raw soya flour. Our results therefore go further than previously reported studies of the influence of pancreatectomy on the development of pancreatic adenomas in rats given azaserine (8) but confirm the effect of partial pancreatectomy in promoting the development of pancreatic cancer in rats given 4-hydroxyaminoquinoline 1-oxide (9) and hamsters given BOP (10).

The augmentation of pancreatic carcinogenesis by partial pancreatectomy resembles the effects of partial hepatectomy on the development of hepatic carcinoma. Partial hepatectomy has been considered to sensitize the liver to weak carcinogens, such as urethane or 2-methyl-4-dimethylaminoazobenzene, which are virtually ineffective against quiescent hepatic tissues (11, 12), just as partial pancreatectomy sensitizes the pancreas to an amount of azaserine which, by itself, does not elicit the development of pancreatic cancer (1).

It seems likely that the augmentation of pancreatic carcinogenesis by partial pancreatectomy is the result of the proliferation of pancreatic acinar cells which results from the pancreatectomy, just as hepatic neoplasms produced by dimethyl nitrosamine and N-methyl-N-nitrosourea develop especially when the genotoxic carcinogen is administered during the peak of the S phase of DNA synthesis and when there is increased mitotic activity (12). The mechanism whereby postresectional cellular proliferation augments carcinogenesis has not been defined, but may result from an increased vulnerability of replicating DNA to damage by carcinogens and to fixation of the abnormal DNA by replication before it is possible to repair the damage to the DNA (11).

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Altered Liver Histology in Patients with Pancreatitis: A Clue to Etiology?

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Abstract

Although commonly regarded as an adaptive response, it is now recognized that chronic induction of cytochrome P450 may have deleterious consequences: our recent work suggests that pancreatitis may be one of these, and we therefore sought to determine, by studying liver histology, whether induction was accompanied by cellular damage. Liver biopsy of 38 consecutive nonjaundiced patients was performed between 10 days and 6 months after an attack of pancreatitis (alcohol 8, miscellaneous 9, idiopathic 21). Altered hepatocytes, occupying 10%–100% of parenchyma, were found in 23 cases (60%). Their appearance varied from large clear cells to typical ground-glass hepatocytes. Toluidine blue staining of ultra-thin sections revealed extensive microvesicular fat in three biopsies; several showed excess lipofuscin and/or steatosis. The portal tracts were altered in 28 cases (74%); changes included diffuse lymphocytic infiltration, focal bile duct proliferation, focal ductopenia, and features suggesting primary sclerosing cholangitis or primary biliary cirrhosis. The spectrum of histological changes was similar in 30 patients with chronic and 8 with acute pancreatitis irrespective of putative etiological agent.

Not only are these hepatocytic changes compatible with an "induction-damage" concept, but the high frequency of portal tract lesions suggests that they too may be similarly initiated, perhaps via biliary excretion of reactive intermediates.

Our recent findings in patients with pancreatic disease—heightened early-phase disappearance of sulfobromophthalein (1), increased bilirubin in bile (2), accelerated clearances of antipyrine and theophylline (3)—are collectively best explained by induction of hepatic enzymes that are directly (cytochrome P450, glutathione S transferase B) or indirectly (heme oxygenase) involved with the "detoxification" of xenobiotics. Although induction of these enzyme systems is generally regarded as nature's protective device, it is known that under certain circumstances, induction may have deleterious consequences, due to reactive metabolites (oxygen-free-radicals, lipid peroxidation products, epoxides, drug radicals, carcinogens) that may be generated via the cytochrome-P450 system (4): the hepatotoxicity of carbon tetrachloride and of acetoaminophen are obvious examples, but ethanol-induced liver damage may also be produced, at least in part, by lipid peroxidative mechanisms (5). Our discovery of increased amounts of lipid peroxidation products in the bile from patients with pancreatic disease (6), together with the high frequency of sclerosing cholangitis-like changes in their retrograde cholangiograms (7), prompted a study of liver histology in these patients.

Patients, Materials and Methods

Patients. Consecutive patients with pancreatitis who required admission during a six-month period had a percutaneous liver biopsy if one or more of the following criteria applied and provided there was no indication for abdominal surgery (gallstones, pseudocyst drainage, biliary diversion, peptic ulcer): hepatomegaly; distorted

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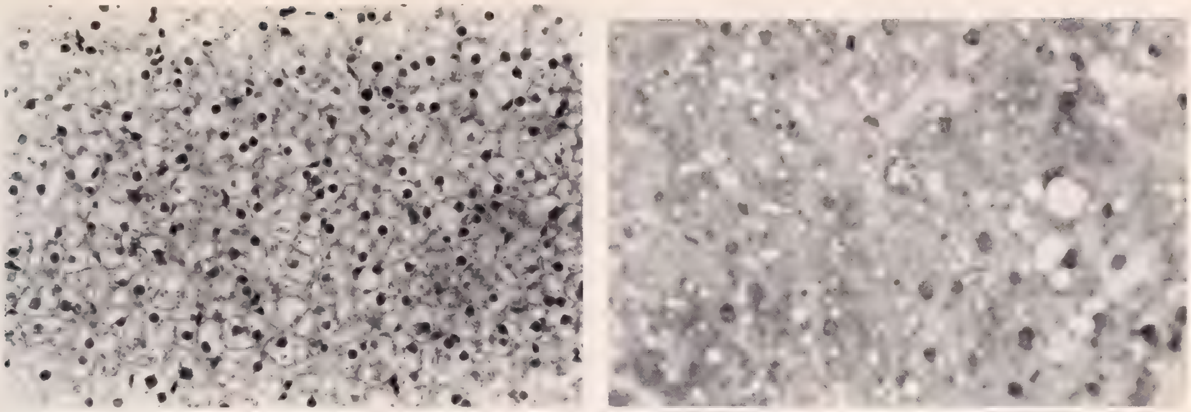
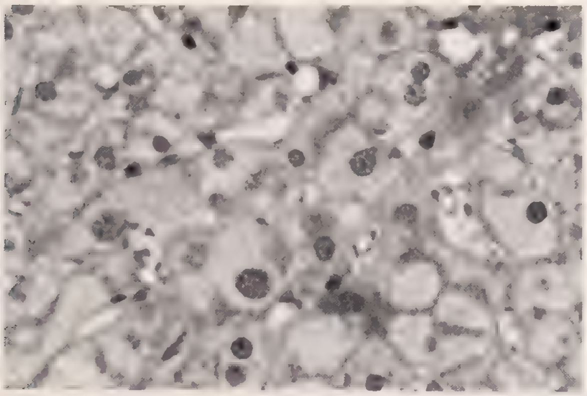


Fig. 1 Patient MP. Left Water clear type I hepatocyte (H & E × 96). **Right** Microvesicular fatty infiltration (toluidine blue ×240).

serum liver function profile; increased lipid peroxidation products in secretin-stimulated bile; sclerosing cholangitis-like changes on retrograde cholangiography. The patency of the biliary tree was confirmed by cholangiography—usually retrograde, occasionally operative or intravenous—in all but two cases: a patient with alcohol-related disease (GH in Table 1) developed jaundice with each attack of pancreatitis and his retrograde cholangiogram showed constriction of the intrapancreatic bile duct; another patient (JH) had a choledochoenterostomy 3 years previously to treat a similar problem. In patients who required surgery, a wedge biopsy of the liver was taken at the start of the operation. A postmortem liver biopsy was obtained from a 49-year-old woman (JS in Table 1) who died from fulminating septicemia 36 hours after ERCP; she had been referred with recurrent attacks of pancreatitis that continued after cholecystectomy (no gallstones) and a retrograde cholangiogram had shown several tight strictures in the intrahepatic ducts indicating sclerosing cholangitis (7).

Twenty-nine patients were classified as having chronic pancreatitis on the basis of abnormal pancreatograms or exocrine function or both (8). The majority had sustained an attack of pancreatitis between 6 weeks and 14 months earlier; two patients had continual pain and 5 had painless steatorrhoea. Nine of these patients had pancreatic calculi demonstrated by a plain X-ray of the abdomen or computed tomography. Two patients with painless pancreatic steatorrhoea, of whom one had pancreatic calculi, had a positive antimitochondrial antibody test (Table 1). Two patients had a long history of ulcerative colitis and were on long-term treatment with Salazopyrin; a patient with Crohn's colitis developed her first attack of pancreatitis 5 weeks after starting treatment with azathioprine. Factors



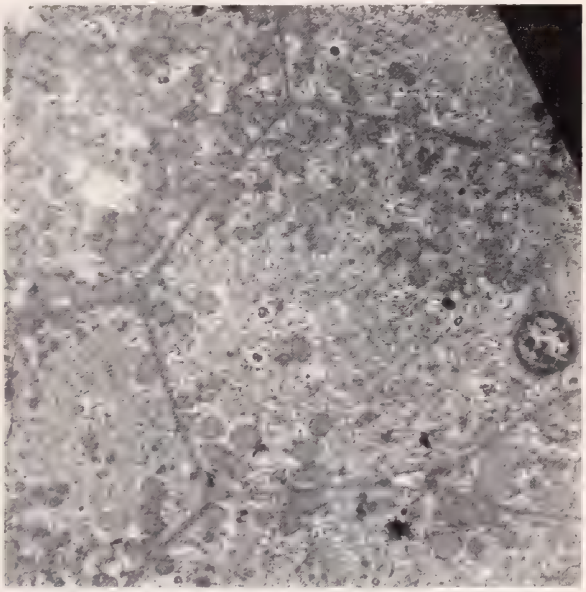


Fig. 2. Patient FP. Group of ground-glass type III hepatocytes (H & E ×240). Electron microphotograph showing extensive margination of mitochondria at periphery of cells due to central proliferation of type II smooth endoplasmic reticulum (uranyl acetate and lead citrate ×2750).

TABLE 1
Clinical Characteristics

						Serum‡				
Pt	Sex	Age	Etiologic factors	Presenting features	Months since last attack	Albumin g/l (38–48)‡	Alanine transferase u/l (40)‡	AP u/l (300)‡	Bil umol/l (20)‡	γGT u/l (65)‡
Chronic										
AB	M	35	alcohol type IV†	RP*	3	42	59	128	11	135
ER	M	47	alcohol	RP*	4	35	88	165	8	71
GH	M	33	alcohol	RP*	2	31	127	159	20	523
DW	M	48	alcohol	Pain*	4	48	53	311	12	268
JP	M	42	alcohol	RP	9	44	28	175	3	116
HB	M	54	alcohol	RP pseudocyst	8	39	14	68	9	13
AS	F	24	alcohol	RP pseudocyst	2	40	28	94	17	30
RS	M	29	alcohol	RP	6	41	66	305	14	184
PB	F	20	idiopathic	RP	6	38	17	71	10	7
MB	F	61	idiopathic	RP*	14	36	38	48	10	29
AP	F	23	idiopathic	RP*	13	43	16	87	1	3
PM	M	34	idiopathic	RP*	2	38	28	164	1	16
FP	M	78	idiopathic	steatorrhoea diabetes	—	41	20	48	14	13
GH	F	34	type IV†	RP	6	35	91	107	21	42
JH	M	49	?Phenobarb	steatorrhoea myelopathy	—	29	400	297	19	1290
BH	M	22	idiopathic	RP	7	39	21	70	15	19
MR	F	54	steroids azathiopine type IV†	epigast pain renal transpl	—	31	20	110	13	32
NE	M	64	idiopathic	steatorrhoea	—	32	35	116	10	450
LM	F	31	azathioprine	RP, Crohn's	6	32	86	50	10	91
MP	M	15	idiopathic	RP	1.5	38	27	105	14	8
CA	M	23	idiopathic	RP	6	40	18	57	16	12
JJ	M	55	idiopathic	RP	4	39	30	86	3	60
JC	M	42	idiopathic	RP	2	27	22	53	10	15
AL	M	41	idiopathic	RP	6	38	25	87	13	10
JS	M	60	idiopathic	Wt loss*	—	31	104	1080	20	393
GK	F	53	idiopathic	RP	4	37	16	77	8	18
PT	F	33	?Salazopyr	ulc colitis steatorrhoea	—	39	111	485	20	180
JJ	M	36	?Salazopyr	steatorrhoea	—	35	94	940	20	500
TL	M	32	idiopathic	RP*	48	40	34	54	7	70
AH	F	62	idiopathic	steatorrhoea itching	—	35	96	888	147	441
Acute										
MC	F	26	contraceptive	1 attack	11	41	25	38	16	88
MC	M	29	idiopathic	RP, diabetes choledochal cyst	24	38	74	96	10	21
AN	M	60	type IV†	1 attack, diabetes	2	41	39	34	16	26
AM	M	43	idiopathic	1 attack pseudocyst	5	31	20	54	15	13
EH	F	50	idiopathic	RP	14	38	21	74	10	60
AH	F	72	gallstones	RP	6	42	14	76	19	110
LH	F	60	gallstones	RP	0.5	32	160	385	21	351
JS	F	40	sulindac indomethacin	RP	1.5	40	65	650	20	183

RP, relapsing pancreatitis.

Prothrombin time was normal in all but one patient (AH, with PBC), in whom it was 20 seconds (normal 14 seconds).

* Pancreatic calculi.

† Type IV hyperlipidemia.

‡ Upper limit of reference range; for albumin, reference range for ambulant patients.

TABLE 2
Histologic Findings

	Parenchyma					
	I (%)	II (%)	III (%)	Total (%)	Miscellaneous†	Portal tracts*
Chronic						
AB	0	0	0	0	Iron +	Lipogranulomatosis
ER	0	0	0	0	Lipofuscin + +, fat +, FN +	Lymphocytes; FP
GH	0	0	0	0	Cholestasis	Large duct obstruction
DW	30	0	10	40	INV +, fat +, lipofuscin +	Lymphocytes
JP	0	20	30	50	—	Normal
HB	0	0	40	40	Lipofuscin + +	Normal
AS	0	10	0	10	Fat +. FN +	PBC (3)
RS	10	0	0	10	—	Lymphocytes
PB	0	0	40	40	Iron +	Normal
MB	30	20	0	50	INV +	Lymphocytes, FP
AP	50	10	0	60	INV +	Normal
PM	0	0	0	0	Iron +, fat +	Lymphocytes, FP
FP	0	20	30	50	Iron +	Lymphocytes
GH	0	0	0	0	FN + +, fat +	Lymphocytes, FP, FD
JH	0	0	0	0	Copper +	?SC ?PBC
BH	0	0	0	0	—	Normal
MR	50	10	0	60	—	Hamartoma
NE	0	0	30	30	INV +	Lymphocytes
LM	0	0	0	0	—	Lymphocytes
MP	100	0	0	100	Microvesicular fat + + +	Lymphocytes
CA	60	40	0	100	Microvesicular fat + + + Lipofuscin +	Normal
JJ	10	0	50	60	Lipofuscin +	Lymphocytes
JC	20	0	0	20	Lipofuscin + +	Lymphocytes, FP, FD
AL	30	40	0	70	Microvesicular fat + + +	Lymphocytes
JS	0	0	0	0	Copper +	PBC (3)
GK	10	20	0	30	Lipofuscin + + +	Normal
PT	0	0	0	0	Cholestasis, INV +	PBC (3)
JJ	0	0	0	0	Cholestasis, INV +	?SC ?PBC
TL	0	0	0	0	Lipofuscin + +	Normal
AH	30	30	0	60	Cholestasis, FN +, Mallory hyaline	PBC (3)
Acute						
MC	0	20	50	70	—	normal
MC	80	0	0	80	—	lymphocytes
AN	20	0	0	20	Fat +, FN +	lymphocytes
AM	0	0	0	0	Fat + +, INV +	lymphocytes
EH	70	20	0	90	Fat + +, INV +	normal
AH	0	0	0	0	Fat +, INV +	SC
LH	60	0	0	60	—	SC
JS	0	0	0	0	Cholestasis	SC

* Hepatocytes: Type I = water-clear cells, Type III = ground-glass cells, Type II = intermediate type.

† Grade and type of parenchymal abnormalities: — = normal; + = mild; + + = moderate; + + + = severe; INV = intranuclear vacuoles; FN = focal necrosis. Portal tracts: FP = focal proliferation of bile ducts; FD = focal ductopenia; PBC = changes suggesting primary biliary cirrhosis, as usually staged (9); SC = suggesting sclerosing cholangitis; ?PBC ?SC = cross-over pattern.

that have been incriminated in the etiology of chronic pancreatitis—alcohol, drugs, hyperlipidemia—were present in about half of the patients, but no factor could be identified in the rest (Table 1). All but two patients were well nourished as judged by body weight, clinical features, morphometry and dietary assessment; patients JH and LM were some 5 kg underweight. One patient (GH, idiopathic) was a strict vegetarian, and another (CA) ate little meat.

Eight patients were classified as having acute pancreatitis because pancreatic exocrine function and structure were normal when assessed at least 6 weeks after the last attack. A factor that might have been relevant in the etiology of pancreatitis was identified in 5 cases.

The patients, with the exception of an obese maturity-onset diabetic (AN) were of normal weight and none admitted to food fads.

Methods. Percutaneous liver biopsies were obtained from 28 patients and operative wedge biopsies from ten patients. Small portions (on average 1 mm) from each end of the specimen were immediately fixed in gluteraldehyde, postfixed in osmium tetroxide, dehydrated in alcohol, and embedded in epon. Semi-thin sections (0.5–1 μm) were stained by toluidine blue and examined by light microscopy and ultra-thin sections were cut for subsequent ultrastructural examination. The rest of the specimen was fixed in buffered formalin for routine histological examination and a battery of special stains—PAS before and after diastase, Perl's stain for iron, untoned reticulin, Shikata stain for HBsAg and copper-binding protein, hematoxylin picro sirius red stain for collagen—were sandwiched between two sections stained with hematoxylin and eosin.

Two pathologists without knowledge of the clinical details examined the biopsies independently and later compared their results to arrive at a combined decision on each case.

Results

Parenchyma. Hepatocytic changes were found in 18 patients with chronic pancreatitis (60%) and five patients with acute pancreatitis (63%). Three types of cells could be distinguished and have been arbitrarily designated as Types I, II, and III.

Type I refers to large cells with central or peripheral nuclei and clear cytoplasm, which were seen in 16 biopsies (42%) and closely resembled those seen in glycogen-storage disease (Fig. 1): in three cases this striking appearance was clearly due to extensive concealed microvesicular fat which was unmasked by staining the semi-thin sections with toluidine blue (Fig. 1).

Type III refers to typical ground-glass hepatocytes which were found in 8 biopsies (21%): large cells, usually with eccentric nuclei, granular eosinophilic cytoplasm, and granular basophilic condensation at the periphery (Fig. 2).

Type II cells seemed to be intermediate between Types I and III, exhibiting some characteristics of each but not identical with either; these were noted in 12 cases (32%). Shikata stain for HBsAg was negative in all cases. There was wide variation in the type and frequency of altered hepatocytes in the different biopsies and an attempt was made to define the extent of the biopsy field occupied by altered hepatocytes in each case. For this purpose, the two pathologists examined each biopsy simultaneously and made a combined decision (Table 2). Other parenchymal changes, such as steatosis, were noted (Table 2). The spectrum of changes was similar in patients with chronic pancreatitis and in those with acute pancreatitis.

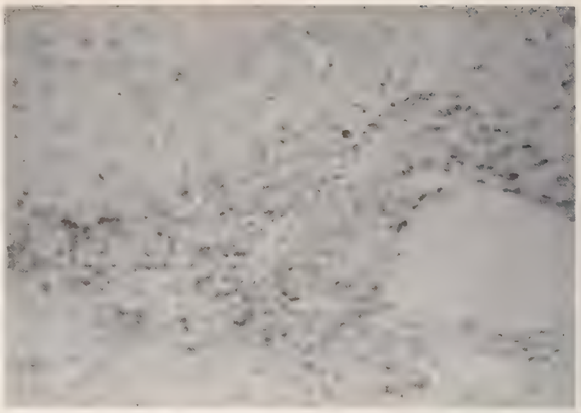


Fig. 3. **Patient PM:** alcohol-related calcific chronic pancreatitis. Portal tract in biopsy shows diffuse lymphocytic infiltration with focal bile duct proliferation.

Portal Tracts. These were normal in 10 patients (26%). Subtle changes consisting of diffuse mild lymphocytic infiltration, usually accompanied by slight expansion of the tracts by fibrosis, were present in 11 cases (19%, Table 2). The portal tracts were unequivocally abnormal in the remaining biopsies (45%). The abnormalities consisted of focal bile duct proliferation (Fig. 3) with (two cases) or without (three cases) focal ductopenia against a background of diffuse mild lymphocytic infiltration in five cases; features highly suggestive of primary sclerosing cholangitis (Fig. 4) in three cases; stage 3 (9) primary biliary cirrhosis in four cases (Fig. 4); an overlap between these entities in two cases; and miscellaneous changes in three patients (Table 2). In the last category were patient GH, with alcohol-related chronic pancreatitis whose liver biopsy strongly suggested large duct obstruction, although he was anicteric at the time; patient AB, also with alcohol-related disease, whose biopsy showed lipogranulomata in the portal tracts; and patient MR, with polycystic kidneys, in whom the only portal tract included in the biopsy showed a hamartoma. The same spectrum of changes was seen in patients with acute or chronic pancreatitis, and there were no obvious differences in the biopsies from patients with alcohol-related or "idiopathic" disease. There were no discernible differences between the primary biliary cirrhosis-like lesions in patients with chronic pan-

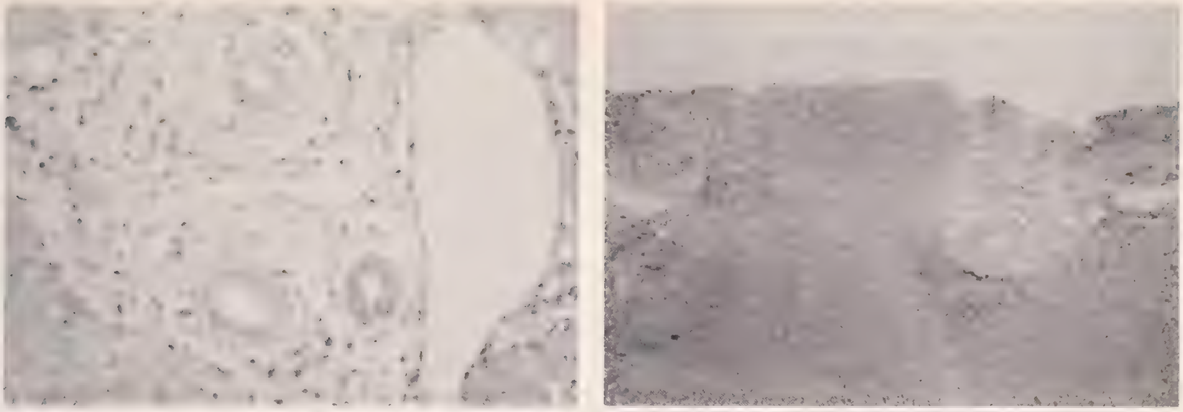


Fig. 4. **Patient AH:** acute pancreatitis (**left**). Typical onionskin appearance of sclerosing cholangitis in portal tract of biopsy. **Patient AS:** alcohol-related chronic pancreatitis (**right**). PBC-like lesion (stage III); AMA negative.

creatitis in whom antimitochondrial antibody was present (AH, JS) and in those in whom it was absent (AS, PT in Table 2). In the three patients with chronic pancreatitis and inflammatory bowel disease the portal tract changes were variable—diffuse lymphocytic infiltration in LM with Crohn's colitis, primary biliary cirrhosis-like changes in PT with quiescent ulcerative colitis, and a crossover pattern between sclerosing cholangitis and primary biliary cirrhosis in JJ, aged 36 years, with severe ulcerative colitis.

Relationship between Parenchymal and Portal Tract Abnormalities. There was a very high incidence of changes in the hepatocytes and/or portal tracts in this study (95%); in a few instances altered hepatocytes occupied the entire field and yet the portal tracts were normal, and vice versa (Table 2). In order to determine whether or not there was any correlation between these histological parameters, the portal tracts were arbitrarily assigned a score of 1 when normal; 2 when there was diffuse mild lymphocytic infiltration; 3 when there was lymphocytic infiltration together with focal bile duct proliferation, with or without ductopenia; and 4 when the changes were highly suggestive of primary biliary cirrhosis or sclerosing cholangitis. The individual scores were compared with the approximate percentage of parenchymal altered hepatocytes in the respective biopsies using the Kendall rank correlation coefficient. An inverse correlation emerged ($r = -0.359$; $p < 0.001$).

Discussion

The voluminous literature on the relationship between hepatic and pancreatic disease has been well summarized by Seeff and Zimmerman (10). Excluding independent effect on each organ by systemic disorders (viral, Kwashiorkor), two main interactions are recognised. First, the hepatic abnormalities have been regarded as a consequence of the pancreatic problem. Thus, steatosis (11), focal necrosis, cholestasis, and infiltration of the portal tracts by inflammatory cells have been reported in up to 80% of patients dying from acute pancreatitis. Since, with the exception of fatty changes, all the other abnormalities occur in the course of experimental acute pancreatitis, it is likely that the hepatic lesions are caused by circulating catalytically active enzymes and vasoactive peptides released from the damaged pancreas (12). Again, among patients with chronic pancreatitis, a substantial number eventually develop hepatic structural and functional changes due to compression of the intrapancreatic portion of the common bile duct, or from concomitant (and possibly secondary) cholelithiasis; cholestasis, cholangitis and secondary biliary cirrhosis have been described in this setting (13–15). Second, the hepatic changes that accompany pancreatitis—whether acute or chronic—have been attributed to the direct and different time-course of the effects of alcohol on liver and pancreas (13, 16).

Our study differs from published reports in that all the liver biopsies were obtained at a considerable interval after an attack (or relapse) of pancreatitis; nonalcoholic patients accounted for the majority; the bile duct was unobstructed in most; and clinical or serological evidence of liver dysfunction was generally unimpressive (Table 1). These differences in patient selection explain why the hepatic parenchymal and portal tract changes which we found have not previously been documented in the context of pancreatitis.

Large hepatocytes, by description very similar to cell type III, have previously been described in patients taking drugs such as phenobarbitone: the ground-glass appearance is due to proliferation of the smooth endoplasmic reticulum (SER, 17, 18). The occasional concurrence of cell types I and II in our study (Table 2) suggests that the various cell types may represent a spectrum, evolving on chronic exposure to a xenobiotic, and our preliminary findings on transmission electron microscopy seem to corroborate this viewpoint (Fig. 2). Indeed, it may be that even those hepatocytes that currently appear "normal" histologically may display ultrastructural aberrations. Of particular importance in this regard is our finding of extensive microvesicular fat in three biopsies (Table 2), a gross disturbance that was only revealed when semi-thin sections were stained by toluidine blue (Fig. 1).

The high incidence of abnormal portal tracts was not entirely unexpected since, with the ready availability of ERCP, associations between biliary and pancreatic ductal abnormalities are increasingly being recognized. Our suggestion that patients with primary biliary cirrhosis frequently have asymptomatic chronic pancreatitis (19) has recently been confirmed (20). Others have arrived at the same conclusion in connection with primary sclerosing cholangitis: a recent report quotes a 77% incidence of abnormal pancreatograms in this condition (21)! On the reverse side of the coin, our recent data show a high incidence of hepatobiliary disease, which is usually asymptomatic, in patients with pancreatitis.

Thus, the spectrum of portal tract changes that we find in patients with pancreatitis is highly reminiscent of the findings of Ludwig et al (22) and Chapman et al (23) in patients with primary sclerosing cholangitis: in both studies, mild to moderate diffuse lymphocytic infiltration of the portal tracts was the commonest abnormality; focal ductopenia, bile ductular proliferation, or periductal fibrosis occurred less often; the typical onionskin lesion of primary sclerosing cholangitis was present in relatively few biopsies; and the appearances were more suggestive of primary biliary cirrhosis in the remainder. This striking histological similarity has a radiologic counterpart, for we have recently reported a high incidence of sclerosing cholangitis-like changes, 83%, in patients with pancreatic disease (7).

We are thus forced to conclude, as others have recently (24, 25), that sclerosing cholangitis-like lesions may be commoner than realized, and that they do not necessarily follow a predictively downhill course. Others have alluded to the covert nature of sclerosing cholangitis in the presence of ulcerative colitis (23, 25); as in that condition, so too in pancreatitis, the presenting features, of rectal bleeding or catastrophic abdominal pain respectively, force the patient to seek medical attention at a time when the liver condition has not advanced sufficiently to cause symptoms or to significantly alter the serum liver function profile.

As in the case of the hepatic functional aberrations we have noted—accelerated clearance of sulphobromophthalein (1), antipyrine and theophylline (3); increased bilirubin in bile (2)—so too in the case of the hepatic histological changes, the similarity in the findings within the different groups of patients (regardless of age, sex, putative etiological agent, interval between clinical attack and biopsy, acute or chronic status of pancreatitis, complicated or not) argues against these hepatobiliary changes being mere coincidences or epiphenomena. Instead, circumstantial evidence favors the possibility that these hepatobiliary changes may provide important clues to the etiology of pancreatitis, and that they may furthermore be linked to microsomal enzyme induction and its consequences.

Thus, alcohol and drugs such as azathioprine and steroids are incriminated in pancreatitis; these agents are also known to induce proliferation of the hepatic SER. Furthermore, microvesicular fat is characteristic of the hepatic damage that may occur in pregnancy or on exposure to oral contraceptives, tetracycline, or sodium valproate—conditions that are also associated with pancreatitis. In the case of sodium valproate, the hepatic changes have been ascribed to a toxic intermediate generated through its oxidation by cytochrome P450 (26). The finding of excessive amounts of lipofuscin in several biopsies (Table 2) is in keeping with this general concept: lipofuscin in tissues is a product of lipid peroxidation (27), which itself is a corollary of increased (?unmitigated) "oxidative detoxification reactions" (28). Indeed, lipid peroxidation has been proposed as a mechanism for alcohol-induced fatty liver (29), and it may therefore be relevant that steatosis was found in so many biopsies. Steatosis, of course, accompanies obesity and severe malnutrition, but in only three of our 38 patients could these factors be invoked. A deficiency of "lipotropic factors" and antioxidants might, however, have contributed to liver damage in the patients with food fads (29). Several products of microsomal metabolism of exogenous and endogenous substrates are excreted in bile (30, 31) including lipid peroxidation products, which have the potential to alter the structure, and thereby the immunogenicity, of gammaglobulin (32).

These various observations suggest that the sclerosing cholangitis-like and primary biliary cirrhosis-like changes which we have found in patients with pancreatitis may also be initiated by excreted toxic reactive intermediates, and the inverse correlation between the hepatocytic and portal tract changes in our study support this idea. The immunologic disturbances and multisystem effects that characterize the classical case of primary sclerosing cholangitis or primary biliary cirrhosis may be secondary to formation of abnormal immune complexes (32) in genetically susceptible individuals (33–36), that is, the portal tract changes may represent a disease spectrum (37).

Our study adds a new dimension to the interrelationship between hepatic and pancreatic disease. The induced liver may be the prime mover in this relationship and reflux of abnormal bile the link (38). Alternatively, the pancreas may be a corecipient of damage initiated by xenobiotics, both identified and unidentified. Whereas the finding of bile pigment in the main pancreatic duct at some distance from the bile duct in surgically removed specimens of pancreas from a few patients with chronic pancreatitis supports the first possibility (38), ultrastructural studies of the pancreas in asymptomatic chronic alcoholics (39), and of uninvolved portions of the pancreas in patients with chronic pancreatitis (40) support the second. The changes in pancreatic acinar cells—expansion and vacuolation of the endoplasmic reticulum, lipofuscin-like inclusion bodies, degranulation of ribosomes and steatosis—so similar to our findings in hepatocytes, are compatible with microsomal induction and its consequences (41). Recent reports show that in most species the pancreas contains phenobarbital-inducible and hydrocarbon-inducible forms of cytochrome P450 (42), making our suggestion, that is, that microsomal induction is the final common pathway for all noxious stimuli to the pancreas, not implausible.

We were at first reluctant to embark on this study, realising that the indications for liver biopsy in some patients were somewhat unconventional, but in retrospect we believe that these "early" hepatic changes have provided valuable insights into events that may be operative in the pancreas itself. This new evidence will, it is to be hoped, provide a more rational approach to treatment of these frustrating diseases.

Acknowledgments

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Increased Bile Pigment Secretion in Nonjaundiced Patients with Pancreatic Disease

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Abstract

A retrospective analysis of data from secretin pancreozymin tests discloses differences between the bile pigment responses of 19 healthy volunteers and 58 nonjaundiced patients with pancreatic disease. The secretion of bile pigment during 30 min after intravenous injection of Boots secretin is significantly higher than normal in 7 patients with acute pancreatitis ($p = 0.009$), 44 patients with chronic pancreatitis ($p < 0.001$), and 7 patients with pancreatic cancer ($p = 0.001$). These findings are unlikely to be due to gallbladder dysfunction, since the pigment responses exceed the values in 10 cholecystectomized individuals ($p < 0.001$). The results suggest induction of proteins that are involved in the production and binding of bilirubin within the liver.

The normal pattern of bile pigment secretion into the duodenum after secretin (1–3) and after pancreozymin (4) has been clearly described by several authors, who have also documented the changes due to gallbladder dysfunction, cholecystectomy, and bile duct obstruction. There is, however, very little information on the bile pigment responses of nonjaundiced patients with pancreatic disease (5, 6). We decided to explore this aspect because of our inexplicable preliminary findings of increased bile pigment after secretin in untreated patients with chronic pancreatitis (6) and our very recent discovery of increased lipid peroxidation products—suggesting hepatic microsomal induction—in the bile from patients with pancreatic disease (7).

Methods

Data from secretin-pancreozymin tests conducted in this department form the basis for this report. The technique was described in detail previously (6, 8). Seven consecutive 10-min collections of duodenal juice are obtained—a basal specimen, three specimens after an intravenous injection of two Crick Harper Raper Units kg^{-1} of Boots secretin, and three specimens after an intravenous injection of 2 CHRu kg^{-1} of Boots pancreozymin. The volumes of successive samples and their pH are measured, and bicarbonate concentration is determined by back titration and the tryptic activity by a pH stat method (9): the outputs of bicarbonate and of enzyme during 30 min after each hormone are calculated. The icteric index (10, 11) provides a simple measure of the concentration of bilirubin (12) in duodenal contents (Fig. 1): the outputs of pigment after each hormone are calculated.

Several studies were omitted at the outset: those performed at a time when the potency of Boots secretin had fallen (13); studies in patients with obstructive jaundice; studies in patients who had been on severely restricted fat intakes and oral pancreatic extracts for at least six months because of the alteration in biliary copper and pigment responses (6); and studies that were technically unsatisfactory. The study group thus consists of 19 healthy volunteers, 44 patients

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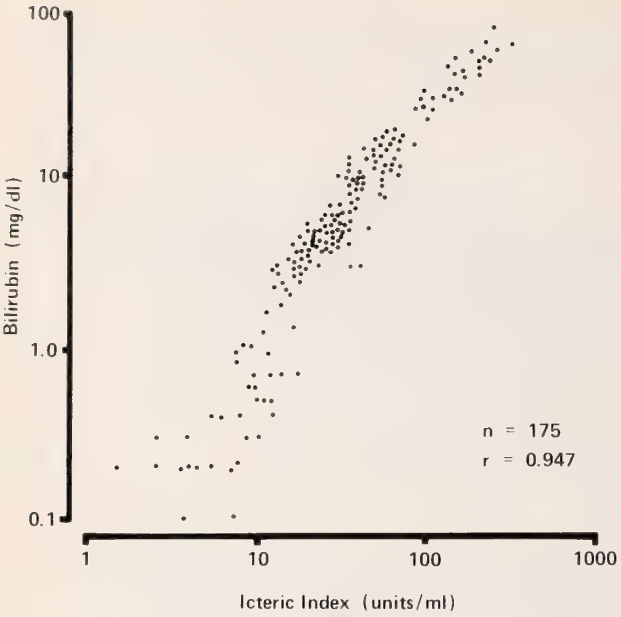

Fig. 1. Correlation between icteric index (11) and bilirubin concentration (12).

with chronic pancreatitis, 7 patients who had recovered from acute pancreatitis, 7 with pancreatic cancer, and 10 who had previously had a cholecystectomy: volunteers were studied in small groups over the years.

The diagnosis of chronic pancreatitis was principally based on histology of previously resected pancreatic specimens in 10 patients, pancreatic calculi demonstrated radiologically in 14 patients, unequivocally abnormal endoscopic pancreatograms in 15 patients, and grossly reduced pancreatic exocrine secretion in 5 patients. Twenty-five of these patients drank alcohol excessively, and in the remainder there was no obvious etiologic factor (two patients had ulcerative colitis but were not on steroids). Six of the patients with chronic pancreatitis had previously had a cholecystectomy. When the endoscopic pancreatogram as well as the pancreatic secretory study were normal, the patient was judged to have acute or recurrent acute pancreatitis (14): the attack (or attacks) of acute pancreatitis appeared to be precipitated by gallstones in one patient, furosemide in another, hypercalcamia in a third, spree drinking in three, and type V hyperlipidemia in the last. None of these patients had previously been cholecystectomized. The diagnosis of pancreatic cancer was confirmed by histology in all patients, none of whom had a previous cholecystectomy. Conversely, in none of the patients in the cholecystectomy group was there any historical, clinical, or biochemical evidence of pancreatic disease. Traditionally, pancreatic secretory studies are delayed until at least six weeks after a clinical attack of pancreatitis so that the extent of pancreatic functional recovery can be assessed.

The results in pairs of subgroups have been compared using Mann Whitney U tests (two-tailed), since the numbers in most subgroups are too small to make any distributional assumptions.

Results

The Normal Response. Injection of secretin provokes an immediate flow of bile, but this effect is transient, so that the material aspirated between 20 and 30 min after injection is virtually colorless (Fig. 2). The volume of duodenal aspirate increases rapidly in the first 10 min and then gradually dwindles: the net result is a fourfold increase in output of bile pigment 10 min after secretin injection (Fig. 3). The cholecystokinetic effect of pancreozymin results in an immediate increase in the flow of bile, resulting in a 10-fold increase in output of bile pigment within 10 min after the injection (Figs. 2 and 3). The response is transient, and basal values are reached some 20 min later. The peak concentration of bicarbonate typically occurs in the third 10-min period after injection of secretin. The magnitudes of the pancreatic enzyme responses to these doses of secretin and pancreozymin are similar, although the response to secretin is probably largely a "washout" phenomenon whereas the response to pancreozymin represents its enzyme-stimulating effect (8).

After Cholecystectomy. Two features are characteristic: there is no increase in bile pigment after pancreozymin, and duodenal secretions tend to remain bile-stained throughout the test, instead of becoming virtually colorless at 30 min as occurs normally (Figs. 2 and 3). The outputs of bicarbonate and trypsin are not significantly different from the values in controls (Table 1).

Acute Pancreatitis. There is a significant increase in the output of bile pigment in each collection period after secretin compared with the values in controls ($p < 0.05$ throughout): the concentrations of bile pigment at 10 and 30 min after secretin are also significantly higher than in controls ($p = 0.030$ and $p < 0.001$, respectively), but bile pigment concentration is not significantly different from that in controls at 20 min after the injection ($p = 0.06$, not significant). That the contractility of the gallbladder is unimpaired after recovery from acute pancreatitis is shown by the

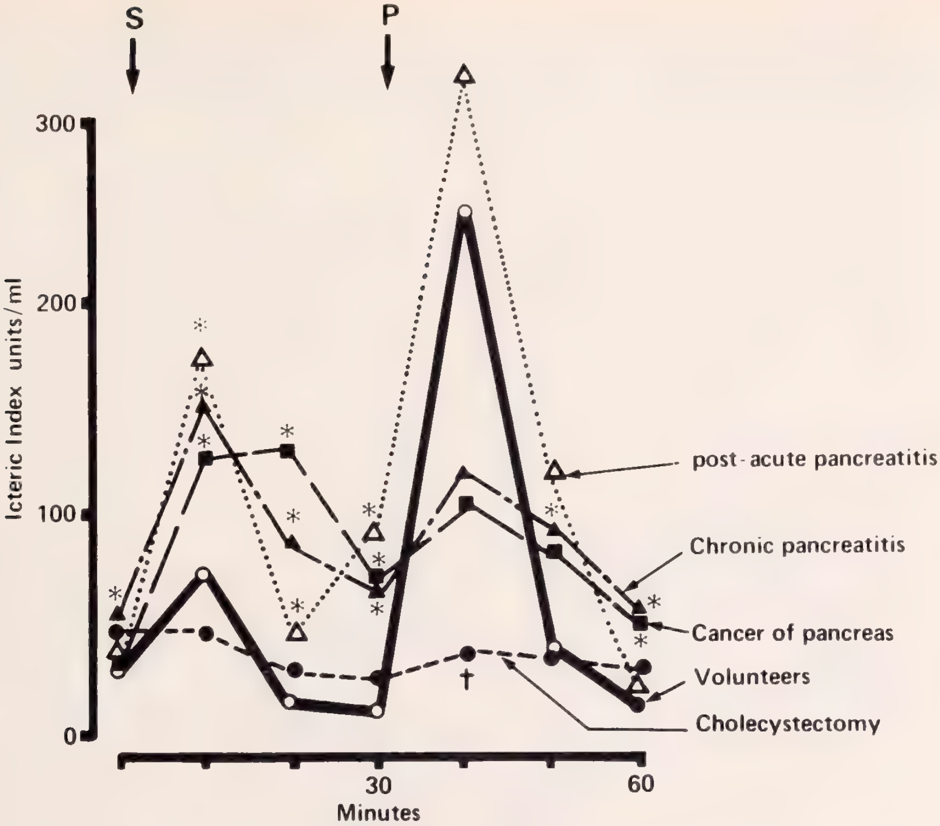

Fig. 2. Icteric index of duodenal secretions collected during secretin-pancreozymin tests in 19 volunteers, 10 cholecystectomized patients, 44 patients with chronic pancreatitis, 7 patients who had recovered from an attack of acute pancreatitis, and 7 patients with pancreatic cancer. S and P, times at which intravenous injections of secretin and pancreozymin are given. Points, medians of values in each group at various time intervals. Asterisks, significant increases in values in various groups compared with values in group of volunteers. Crosses, significant reductions in values compared with values in group of volunteers.

normal bile pigment response to pancreozymin (Figs. 2 and 3 and Table 1).

Chronic Pancreatitis and Pancreatic Cancer. In both groups the concentrations and amounts of bile pigment after secretin are significantly higher than the values in controls (Figs. 2 and 3: $p < 0.001$ for each comparison in chronic pancreatitis; $p < 0.01$ for each comparison in pancreatic cancer with the exception of the amount of pigment in the first 10 min, $p > 0.10$). In both groups the response to pancreozymin is delayed, compared with the normal pattern (Figs. 2 and 3). The concentration of bile pigment is lower than normal soon after the injection but higher than normal thereafter (differences significant only at 50 and 60 min in chronic pancreatitis and 60 min in pancreatic cancer; $p < 0.005$ for each comparison). The peak outputs of pigment are significantly reduced ($p < 0.05$ for each), but because of the increased outputs in the later periods ($p < 0.05$ for each period in chronic pancreatitis; $p < 0.10$ for each period in pancreatic cancer), the total outputs of pigment at 30 min after pancreozymin are not significantly different from the values in controls (Table 1).

Variation in Bile Pigment Response. Two factors contributing to the wide variation in bile pigment responses to secretin (Table 1) have been identified by consecutive secretory studies in the same patients but under different circumstances; examples are given in Table 2. A 22-year-old patient with idiopathic, recurrent, noncalcific chronic pancreatitis (diagnosed by endoscopic pancreatography and reduced pancreatic secretory parameters) was studied six weeks after an acute relapse (May 1983) and on a second occasion three days after the next relapse (July 1983). There is a 10-fold increase in the bile pigment responses to secretin and to pancreozymin in the second study compared with the respective responses in the first: the reduction in enzyme secretion in the second study is expected because of

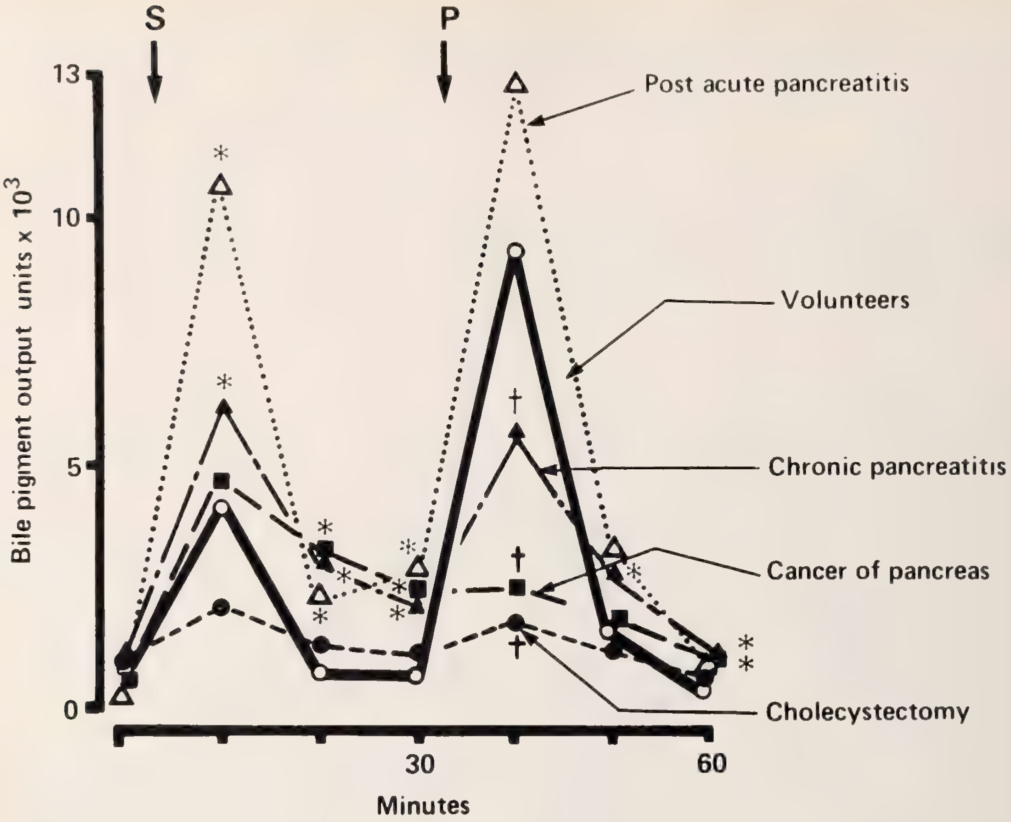

Fig. 3. Outputs of bile pigments in secretin-pancreozymin tests in five groups of individuals. Symbols as in Fig. 2.

the recent pancreatic inflammation (Table 2). Similar data are available in three other patients. By contraast, in a 56-year-old patient with recurrent acute pancreatitis and hypertriglyceridemia, the bile pigment response after 12 months on a low fat intake (40 g/day with virtual removal of polyunsaturated fat) is substantially reduced whereas the outputs of bicarbonate and

TABLE 1

*Median Values and Ranges for Outputs of Trypsin, Bicarbonate, and Bilirubin 30 min after Secretin and 30 min after Pancreozymin in Various Study Groups**

	Responses to secretin			Responses to pancreozymin		
	Trypsin (units/30 min)	Bicarbonate (mmol/30 min)	Bile pigment (units/30 min)	Trypsin (units/30 min)	Bicarbonate (mmol/30 min)	Bile pigment (units/30 min)
Volunteers (19)						
Median	3220	8.27	5,705	5.640	9.47	11,728
Range	1,764–8,622	4.45–17.00	493–14,186	1490–11085	3.88–16.90	2,170–27,792
Cholecystectomy (10)						
Median	4461	9.00	4,084	5,164	11.33	4,506
Range	3,528–10,759	5.87–20.92	1,086–11,896	3,066–9,329	4.50–22.77	1,592–6,522
Significance	NS	NS	NS	NS	NS	$p < 0.001$
Acute pancreatitis (7)						
Median	4738	7.07	16,722	5,093	8.89	18,475
Range	2,753–7,081	3.07–11.25	4,518–30,324	3,044–11,678	5.78–12.57	4,395–51,158
Significance	NS	NS	$p = 0.009$	NS	NS	NS
Chronic pancreatitis (44)						
Median	2393	3.49	10,707	1,723	3.29	10,317
Range	0–9,752	0.26–13.37	4,902–27,966	0–8,225	0.14–14.10	1,279–80,350
Significance	$p = 0.021$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	NS
Cancer of pancreas (7)						
Median	3,991	5.19	10,189	2,230	4.11	6,187
Range	396–7,184	0.72–11.42	8,288–30,685	90–6,125	0.14–11.47	1,196–12,950
Significance	NS	$p = 0.041$	$p = 0.001$	$p = 0.022$	$p = 0.022$	NS

* Significance of differences between test and control groups is assessed by Mann Whitney U tests (two-tailed).

trypsin are higher (Table 2). Although this patient used to have very frequent attacks of pancreatitis, he has been asymptomatic for the past six months.

Discussion

Duodenal intubation studies with hormones are traditionally used to assess the functional state of the pancreas, and there are many versions of these tests. The two prerequisites are that the doses of secretin and pancreozymin should evoke near-maximal responses of bicarbonate and pancreatic enzyme, respectively—as is the case in our version (13)—and that duodenal secretions are completely recovered. To ensure the latter, some advocate the routine use of nonabsorbable markers, but in our experience, as in that of others (15), this maneuver may distort the picture and grossly overestimate secretory rates. Provided that the test is performed meticulously, the results in the simple secretin pancreozymin test are comparable to those derived from more cumbersome marker dilution studies (15): it is probably valid to conclude that although the former may underestimate secretory rates, it is impossible to overestimate these; the reverse is true with studies using markers.

The pioneer workers in this field appreciated that valuable information on the biliary tract is simultaneously obtained during secretin or secretin pancreozymin tests (1–4). Bile staining of the earliest secretions after secretin is ascribed to the "choleretic" effects of the hormone and the transient nature of this phase to the ability of the normal gallbladder to distend and accept secretions from the liver, at a time when the sphincter of Oddi is closed. The cholecystokinetic effect of pancreozymin leads to an abrupt increase in the amount of bile pigment recovered from the duodenum. Disease of the gallbladder results in reduction in the postpancreozymin response and in persistence of bile staining throughout the period after secretin: cholecystectomy magnifies these differences. Thus, the main value of the bile pigment responses has hitherto been to detect gallbladder dysfunction or obstruction of the bile duct (when secretions are colorless throughout). With the ready availability of ultrasound, these indirect assessments are no longer necessary, and the bile pigment responses to secretin and pancreozymin have largely been ignored in recent reports.

An increase in bile pigment secretion after secretin, as in our patients with all types of pancreatic disease, has not previously been documented. This increase cannot be explained by gallbladder dysfunction, which is the likely cause for the reduced bile pigment responses soon after pancreozymin in chronic pancreatitis and pancreatic cancer (Figs. 2 and 3 and Table 1) and may reflect the organ's refractoriness in the face of a chronic excess of circulating hormone (16)—since the peak pigment response to secretin is characteristically normal or reduced after cholecystectomy (Figs. 2 and 3 and Table 1). The finding cannot be dismissed as due to technical artifacts, traces of pancreozymin in Boots secretin, variable "washout" of bilirubin, or spontaneous contraction of the gallbladder because these considerations should apply equally to the group of volunteers and thus reduce, rather than amplify, differences in pigment secretion between the subgroups. It seems more likely that the increased biliary pigment after secretin is a manifestation of altered bilirubin metabolism within hepatocytes, and that this was only revealed because of our fortuitous use of the impure preparation, Boots secretin. It has been suspected for some time that a substance present in intestinal

TABLE 2

Outputs of Bile Pigment, Bicarbonate, and Trypsin 30 min after Secretin and 30 min after Pancreozymin in Two Patients, Each Tested on Two Separate Occasions

	Responses to secretin			Responses to pancreozymin		
	Bile pigment (units/30) min)	Bicarbonate (mmol/30 min)	Trypsin (units/30 min)	Bile pigment (units/30 min)	Bicarbonate (mmol/30 min)	Trypsin (units/30 min)
PtBH						
idiopathic, recurrent, chronic pancreatitis						
6 weeks after acute relapse	10,664	3.86	967	7,908	3.92	794
3 days acute relapse	118,732	2.25	564	119,414	5.31	557
PtHC						
recurrent, acute pancreatitis with type V hyperlipidemia						
6 weeks after acute relapse	32,744	7.26	5125	28,109	8.88	5,903
Quiescent phase after 12 months on low-fat diet	6,395	9.38	9614	10,554	11.83	6,558

extracts of secretin promotes the transfer of hepatocytic products across the canalicular membranes (17). Recently, Romanski and Bochenek (18) showed that a contaminant in Boots secretin —other than glucagon, pancreozymin, or bile salts—has this property, evoking the secretion into bile of phospholipids, cholesterol, and bile acids. The same contaminant may be responsible for secretion into bile of other products of microsomal metabolism including hexauronic acid (17), lipid peroxidation products (7), and bilirubin.

Theoretically (19), our findings could be due to an increase in the bilirubin load presented to hepatocytes, as in hemolytic states; increased hepatic uptake of bilirubin due to an increase in hepatic blood flow; reduced efflux of bilirubin from and thus a heightened accumulation in hepatocytes due to induction of glutathione-S-transferase B (ligandin) and other binding proteins; or an increase in the fraction of bilirubin that is derived from hepatic heme—variously estimated as between 23% and 37% (20–22).

In none of the patients in this study was there any clinical or hematologic evidence of hemolysis (serum total and unconjugated bilirubin, urobilin, reticulocyte count, haptoglobins) or any clinical features to suggest enhanced hepatic blood flow. Induction of hepatic proteins that are involved in the production and binding of bilirubin within the liver seems a more likely explanation for several reasons. First, the high concentrations of lipid peroxidation (free-radical oxidation) products in bile from patients with pancreatic disease strongly suggest induction of the hepatic mono-oxygenases (7, 23). Second, the enhanced net uptake of bilirubin by isolated perfused livers of phenobarbitone-treated rats is clearly due to induction of ligandin, which, by binding bilirubin, reduces its efflux from hepatocytes (24): ligandin also binds other organic anions such as sulfobromophthalein, and induction of ligandin probably explains the heightened early-phase clearance of this dye in patients with pancreatic disease (25). Third, it is well known that stimulation of the heme synthetic pathway is one of the earliest events following administration of "inducing" agents, such as phenobarbitone, to rats (26). The increase in hepatic heme generates more heme-containing microsomal enzymes such as cytochrome P-450 and heme oxygenase: the latter serves to eliminate excess intracellular heme by catalyzing the formation of bilirubin (27). Finally, the accelerated clearance of antipyrine in patients with acute pancreatitis, chronic pancreatitis, and pancreatic cancer (28), taken in conjunction with the frequent occurrence of ground-glass hepatocytes in liver biopsies from these patients (29), confirms induction of the hepatic mono-oxygenases. Increases in ligandin, heme oxygenase, and glucuronyl transferases, consequent on induction, should ensure the prompt disposal and discharge of bilirubin conjugates into bile.

Is the increased bile pigment secretion in our patients a primary event or secondary to pancreatic disease? The similarity in the various hepatic functional changes (6, 7, 25, 28, Table 1) in patients with three histologically distinct forms of pancreatic disease argues strongly in favor of the first possibility. If the increased bilirubin secretion is secondary to reduction in pancreatic exocrine secretion, one might expect inverse correlations between these parameters. That this is not the case is evident from the responses of patients with acute pancreatitis in whom bile pigment secretion is highest, though, by definition, pancreatic secretory parameters are normal (Table 1): variable gallbladder function may explain why the higher postpancreozymin pigment responses in patients with acute pancreatitis do not achieve statistical significance. Further support for this interpretation comes from the responses of patient BH (Table 2): the 10-fold increase in bilirubin output soon after an acute attack is disproportionate to the small reduction in pancreatic secretory constituents.

Our current studies are directed toward elucidating the cause of hepatic "induction" in our patients. In some, alcohol is the obvious explanation; in others, excessive amounts of dietary polyunsaturated fat and dietary inducers together with social or occupation inducers might be relevant (30). Reduction in dietary fat intake, and particularly in the intake of polyunsaturated fats—which exerts a "permissive effect" on the induction process (31)—is associated with a marked reduction in bilirubin output after secretin (HC, Table 2), a phenomenon we had previously observed but misinterpreted (6).

Our deductions regarding the source of the increased bile pigment after Boots secretin must clearly be tested by directly investigating the contribution of "early bilirubin" to total bilirubin in untreated patients with pancreatic disease (20).

Acknowledgments

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Acute Pancreatitis: Update 1986

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The definition and classification of "acute" and "chronic" pancreatitis have been the subject of much debate. The First Marseilles Conference on the Classification of Pancreatitis, in 1963, sought to clarify the application of these terms (1). In 1984, the Second Marseilles Symposium attempted to refine and clarify the classification of pancreatitis in view of more current and sophisticated diagnostic modalities that had become available (2–4). Nevertheless, the original Marseilles terminology abounds in the literature, having persisted for over 20 years. Thus, although we here use the current classification scheme, other terms will be encountered in the literature.

Acute and chronic pancreatitis are defined and distinguished on the basis of clinical, functional, and morphologic criteria. Acute pancreatitis is an isolated episode of symptoms resulting from intrapancreatic activation of digestive enzymes. There is no permanent functional damage to the pancreas. Morphologically, the organ returns to normal following the discrete episode. Only rarely does acute pancreatitis progress to chronic pancreatitis. In contradistinction, chronic pancreatitis is defined as a chronic pain syndrome with progressive, functional impairment resulting in permanent morphologic changes in the pancreas. Acute pancreatitis may recur, and many patients with chronic pancreatitis may have acute exacerbations.

Incidence. In both Rochester, Minnesota and Great Britain, the incidence of acute pancreatitis was approximately 10 per 100,000 population with a mortality of 1.0 per 100,000 (5). The incidence rises progressively from age 30 through the seventh decade, with a median age of 53, and the male-to-female ratio is virtually equal (6).

Pathogenesis. Acute pancreatitis is a discrete episode of symptoms resulting from autodigestion that is triggered by premature transformation of inactive zymogens to active enzyme in the pancreas itself. The events leading to this premature activation of zymogens are unknown. However, several initiating events have been proposed, including: (1) outflow obstruction of pancreatic secretions (7); (2) reflux of duodenal contents through sphincter of Oddi into the pancreatic duct (8); and (3) the "common channel theory of Opie" with bile reflux into the pancreatic duct resulting from gallstone obstruction of the ampulla of Vater (9). Despite much controversy surrounding the etiologic hypotheses, it appears that the autodigestive process itself is initiated by minute amounts of trypsin, which activates the proenzymes of phospholipase A and elastase. The former enzyme causes pancreatic adipose and parenchymal necrosis whereas the latter enzyme causes dissolution of blood vessel elastic fibers (10).

Pathology. The histologic changes of acute pancreatitis are proteolytic disruption of pancreatic substance, blood vessel necrosis leading to hemorrhage, fat necrosis by lipolytic enzymes, and an inflammatory response (11). The actual morphologic changes depend on the duration and extent of the disease process. Initially, only interstitial edema is seen (acute edematous pancreatitis), which may be followed by focal and confluent areas of frank necrosis of both endocrine and exocrine tissue (necrotizing pancreatitis). Blood vessel disruption leads to hemorrhage (hemorrhagic pancreatitis). Patchy areas of inflammation and necrosis alternate with zones of preserved pancreatic tissue (a phlegmon). When the inflammatory response involves the peritoneum,

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"pancreatic ascites" results. When acute inflammation and necrosis begins to subside, fibrosis of parenchyma may ensue. When these liquefied regions are walled off by fibrous tissue, they are called "pseudocysts." If these areas of necrotic and liquefied tissue become colonized with enteric flora, an infected pseudocyst or abscess may form.

Precipitating Factors. Although the exact pathogenesis of acute pancreatitis remains uncertain, multiple etiologies have been identified (Table 1).

Gallstones. Gallstones are the condition most commonly (55%) associated with acute pancreatitis in private hospitals, and the second most common (30%) in city hospitals (12). This disease carries an 11% mortality during the first episode, but mortality declines dramatically with subsequent episodes (13). If gallstones are not removed, 50% of patients will have recurrent episodes of acute pancreatitis (14).

Alcoholism. Alcohol abuse is the most common cause of acute pancreatitis in public hospitals and the second most common in private institutions. Mortality is less than with gallstone-associated acute pancreatitis, partly because these patients are younger and many may have underlying chronic pancreatitis (12, 13). Although the implication in the past has been that alcohol abuse leads only to chronic pancreatitis, it is now known that alcohol-associated pancreatitis can manifest acutely and that alcohol-induced disease is not inevitably progressive (2). When alcohol-induced disease presents acutely, any and all of the complications associated with gallstone and idiopathic pancreatitis may ensue.

TABLE 1

Conditions associated with Acute Pancreatitis

90% of all cases
Gallstones (#1 in private hospitals)
Alcoholism (#1 in public hospitals)
Idiopathic (up to 30%)

Other 10%
Medications (especially sulfonamides and antimetabolites)
Postoperative (abdominal, cardiac surgery)
Trauma (#1 in young people)
Post ERCP (1% of ERCPs)
Hyperlipidemia (types I, IV, and V)
Hypercalcemia (hyperparathyroidism, myeloma, TPN)
Pancreas divisum
Renal transplantation
Cardiac transplantation
Infectious agents
Pregnancy (90% gallstone-associated, third trimester)
Scorpion bite (Trinidad, W.I.)
Ampullary disease
Hereditary
Penetrating duodenal ulcer
Connective tissue disorders with vasculitis
Eating disorders (anorexia nervosa, bulimia)

Idiopathic Factors. Routine evaluation of patients with acute pancreatitis fails to identify an associated condition in up to 30% of patients (4). However, it has long been recognized that at least some of these patients may have radiographically undetectable gallstones (15). Recently, Feller studied 73 patients with "idiopathic" pancreatitis by endoscopic retrograde cholangiopancreatography (ERCP) and found that 23 had potentially curable causes of pancreatitis: 9 had biliary calculi, 5 stenosis of the ampulla of Vater, 3 nonampullary strictures of main pancreatic duct, 5 pancreas divisum, and 1 pancreatic carcinoma (16). Therefore, as our sophistication increases in the recognition of factors that may cause pancreatitis, the percentage of cases termed "idiopathic" will decline.

Medication. Multiple medications have been associated with acute pancreatitis (17). Those believed to have a definite causal relationship include azathioprine, hydrochlorothiazide, furosemide (Lasix), sulfonamides, estrogens, tetracyclines, and valproic acid. Other drugs that may be associated with acute pancreatitis include chlorthalidone, ethacrynic acid, L-asparaginase, phenformin, methyldopa (Aldomet), and corticosteroids.

Postoperative Complication. Traditionally, it has been taught that in from 6% to 13% of all patients with pancreatitis, the condition will occur after operation (18, 19). This complication was noted to have a high mortality rate (25%–42%) and generally followed upper abdominal surgery. Local trauma was felt to play a major role in the pathogenesis of this complication. In recent years, pancreatitis following cardiopulmonary bypass has become increasingly recognized (20–23). Pancreatitis following cardiac surgery is generally severe and associated with a high mortality rate (up to 63%) despite aggressive therapy (24). Possible etiologic factors include prolonged cardiopulmonary bypass with low cardiac output states, leading to hypoperfusion and ischemia of the pancreas.

Trauma. Traumatic injury to the pancreas is the most common cause of pancreatitis in young people. Both penetrating injuries and blunt trauma (from automobile steering wheels and bicycle handlebars) have been implicated (25).

Post ERCP Complication. Approximately 1% of ERCP studies are complicated by development of pancreatitis, which is generally mild and becomes clinically evident within 24 hr (26).

Acute pancreatitis following ERCP is probably related to several iatrogenic factors intertwined with injection into the pancreatic duct, including the speed, volume, and pressure of injection, material injected, number of injections, and the underlying pancreatic condition.

Hyperlipidemia. Patients with Fredrickson types I, IV, and V hyperlipoproteinemia may develop acute pancreatitis (27). Sometimes pancreatic insufficiency develops after multiple attacks, over a period of years (27a).

Hypercalcemia. In 1962, Minter et al reported 62 cases of pancreatitis occurring in association with hyperparathyroidism. It was concluded that pancreatitis was a relatively frequent complication of hyperparathyroidism and thus was an important clue to the diagnosis of that condition (28). In 1980, however, Bess et al reported 1,153 patients with surgically confirmed primary hyperparathyroidism operated on at the Mayo Clinic between 1970 and 1975 (28a). Only 17 (1.5%) had coexisting or prior pancreatitis. This frequency of association approximates the reported incidence of pancreatitis among the general hospital patient population. Other factors of possible etiologic significance in pancreatitis, such as gallstones or alcohol abuse, were present in 11 of the 17 patients. Cure of the hyperparathyroidism was usually not associated with amelioration of symptoms due to pancreatitis. Thus, this study did not support a cause-and-effect relationship between primary hyperparathyroidism or hypercalcemia and pancreatitis. Hypercalcemia of any cause, including such uncommon entities as familial hypocalciuric hypercalcemia (29) and patients receiving total parenteral nutrition (30), may lead to acute pancreatitis.

Pancreas Divisum. Embryologic failure of fusion of the ventral and dorsal pancreatic ducts has recently been associated with pancreatitis (31). It is believed that the orifice of the accessory duct of Santorini, which, with malfusion, drains the major part of the pancreas, does not allow adequate dorsal duct drainage of secretions, thus causing pancreatitis. However, recent evidence suggests that pancreas divisum should not be regarded as an etiologic factor in pancreatitis but should be considered as a coincidental anatomic variant encountered in nearly 10% of the population (31a).

Renal Transplantation. Renal transplantation is associated with a 2%–7% incidence of acute pancreatitis that may progress to chronic pancreatitis (32). The factors that may contribute to this association include immunosuppressive agents such as azathioprine and L-asparaginase, secondary hyperparathyroidism, vasculitis, and viral infections.

Cardiac Transplantation. Acute pancreatitis is being increasingly recognized after cardiac transplantation. The possible etiologies include immunosuppressive agents, viral agents, and hypotension induced during the operation itself (33).

Infectious Agents. This category of etiologic agents is the most infrequently recognized, because they are not considered as inciting causes. Infection with mumps (34), Coxsackie B (35), cytomegalovirus, Epstein-Barr, and hepatitis (36, 37) viruses has been associated with acute pancreatitis. Other infectious agents implicated etiologically include the fluke of *Clonorchis sinensis, Ascaris* worms (38), *Mycoplasma* (39), *Camphylobacter* (40), and legionnaires' disease.

Pregnancy. Ninety percent of patients with pregnancy-associated pancreatitis have concomitant gallstones (12). These episodes occur late in the pregnancy or in the postpartum period, which may be related to the lithogenic effects of pregnancy (41, 42). The prognosis is good with surgical treatment of the associated stone disease. Hyperlipidemia may be associated with pregnancy and may cause pancreatitis.

Scorpion Bite. In Trinidad, West Indies, scorpion bites may lead to mild pancreatitis (43). The underlying mechanism involves release of acetylcholine (44).

Ampullary Disease. Pathologic processes that affect the ampulla of Vater may cause mechanical obstruction to the outflow of pancreatic secretions and, therefore, cause pancreatitis. These conditions include primary duodenal disease such as duodenal duplication (45), trichobezoar (46), Hodgkin's and non-Hodgkin's lymphoma (47), Crohn's disease, periampullary diverticula, and afferent loop obstruction following gastrojejunostomy. Primary ampullary disease such as hypertensive sphincter (48), ampullary tumors (49), and annular pancreas (50) can cause pancreatitis.

Heredity. Hereditary pancreatitis is inherited genetically as an autosomal dominant condition with incomplete penetrance. It has been documented in at least 19 families (51, 52). This form of acute pancreatitis usually begins in the teenage years, may progress to chronic pancreatitis, and has been associated with an increased incidence of pancreatic adenocarcinoma.

Connective Tissue Disorders with Vasculitis. Systemic lupus erythematosus, necrotizing angiitis, and thrombotic thrombocytopenia purpura may be associated with acute pancreatitis. This is most likely on an ischemic basis.

Eating Disorders. Anorexia nervosa and bulimia have been associated with acute pancreatitis (53). The postulated mechanism is that cyclical fasting and binge eating may cause acinar destruction and incite acute pancreatitis via significant variation of pancreatic secretory output.

Clinical Manifestations. Abdominal pain, midepigastric and periumbilical with radiation to the back, is the hallmark symptom of acute pancreatitis (54, 55). The pain ranges from mild to severe and frequently is more intense in the supine, than sitting, position. Abdominal distention is often present with diminished bowel sounds. Nausea and vomiting are common symptoms. Temperature elevation to 100° or 101°F may be present; if the temperature is above this level, sepsis should be considered. The pathologic process is a retroperitoneal burn with secondary volume loss leading to hypotension with circulatory shock. This occurs in 40% of patients. Uncommonly, hypocalcemia, subcutaneous nodules secondary to fat necrosis, and hemorrhagic bluish discoloration of the flanks (Grey Turner's sign) and periumbilical regions (Cullen's sign) are present. Subsequently, the complications of phlegmon, pseudocyst, or abscess cause palpable abdominal masses in up to 15% of patients (56).

Mild jaundice may be present, secondary to encroachment upon the distal common bile duct by an inflamed pancreas. This inflammation may extent to the duodenal C loop and stomach leading to minor mucosal bleeding in 10%–20% of patients. Paralytic ileus develops in 10% of patients. Pulmonary complications with marked hypoxemia occur in a significant percentage of patients. These complications include pleural effusions, elevated diaphragms secondary to an ileus, lower-lobe infiltrations, and impaired ventilation with arterial hypoxemia (57). Although abdominal pain is the hallmark of pancreatitis, painless acute pancreatitis occurs in up to 2% of cases. In these episodes the presentation is often shock or coma and the prognosis is extremely poor (58).

Laboratory Features. Mild elevation of the serum amylase level is a sensitive but not specific test for the diagnosis of acute pancreatitis. However, levels greater than five times normal are infrequently of extrapancreatic origin (58a). Serum amylase levels generally become elevated early in the course of acute pancreatitis and remain elevated for 24–72 hr. The magnitude of the enzyme elevation is unrelated to the severity of the pancreatitis. In fact, a normal serum amylase occurs in 10% of lethal pancreatitis (59). Thus, although the magnitude of the amylase elevation is not correlated with the severity of pancreatitis, the height of the amylase is useful to differentiate pancreatic from nonpancreatic origin.

Serum amylase can be elevated in a variety of illnesses that simulate acute pancreatitis (60, 61). The causes of elevated amylase not related to the pancreas (Table 2) include biliary colic, perforated peptic ulcer, mesenteric infarction, salivary gland dysfunction, renal insufficiency, macroamylasemia, and tumors (especially lung and ovary). To differentiate pancreatic from nonpancreatic sources of serum amylase, isoamylase determinations have been developed (62, 63). In normal individuals, approximately 40% of total serum amylase is of pancreatic origin (P type) and 60% of total serum amylase is of salivary origin (S type). In acute pancreatitis, the majority

TABLE 2

Nonpancreatic Conditions associated with Increased Serum Amylase Activity

Intraabdominal diseases
- Biliary tract disease, including acute cholecystitis and spasm or stenosis of the sphincter of Oddi
- Perforation of esophagus, stomach, small intestine, or colon
- Intestinal ischemia or infarction
- Intestinal obstruction
- Appendicitis, acute
- Diverticulitis, acute
- Gynecologic conditions, acute (salpingitis, ruptured ectopic pregnancy)

Abnormalities of salivary glands
- Mumps
- Calculous obstruction of salivary glands
- Scorpion sting
- Effects of alcohol

Tumors
- Lung carcinoma
- Papillary cystadenocarcinoma of the ovary
- Ovarian cysts

Renal insufficiency

Macroamylasemia

Miscellaneous
- Head trauma with intracranial bleeding
- Diabetic ketoacidosis
- Anorexia nervosa
- Prostatic disease
- Peroral endoscopy
- Drugs (morphine, for example)
- Pneumonia

of serum amylase is of the P type. In other diseases that elevate total serum amylase levels, S type isoamylase is elevated. However, in other abdominal illnesses, such as perforated peptic ulcer and mesenteric infarction, P type isoamylase is the only type of amylase released from the intestine and found in the serum (61). Thus, although isoamylase determinations appear to be of some clinical value, they probably cannot distinguish between acute pancreatitis and acute abdominal surgical catastrophes.

Serum lipase levels are usually elevated in acute pancreatitis and, along with pancreatic isoamylase, remain elevated longer than total serum amylase. Thus, both pancreatic isoamylase and lipase may be useful in evaluation of pancreatitis that initially presents in the recovery phase. However, lipase may be more useful than pancreatic isoamylase because in macroamylasemia the former remains normal but the latter is elevated (64). Urine amylase determination has a very limited role for diagnosing pancreatic disease. The urine amylase clearance is increased in patients with acute pancreatitis and may remain elevated for 9–15 days, but is also increased in tubular disease states, i.e., diabetic ketoacidosis, burns, neoplasms, renal failure, chronic hemodialysis and fulminant alcoholic liver disease. Factitious pancreatitis, an entity in which the patient expectorates into his urine sample and fakes abdominal pain, also may produce elevated urine amylase (66). In summary, these determinations are of little value to the clinician.

Hyperlipidemia, types I, IV, or V, with serum triglyceride levels exceeding 1,700 mg/dl may be present in acute pancreatitis. Hyperlipidemia may interfere with the laboratory determination of amylase. This inhibition can be overcome by serum serial dilution.

Nonspecific, but associated, laboratory features of pancreatitis include leukocytosis (usually 10,000–25,000 per mm^3), hypocalcemia (in up to 30% of cases), hypoproteinemia and hypoalbuminemia, elevated hematocrit (due to hemoconcentration), and decreased arterial pO_2.

Radiographic Abnormalities. The flat plate of the abdomen may reveal (a) a sentinel loop (a dilated jejunal loop adjacent to the pancreas); (b) the colon cutoff sign (air-filled transverse colon without colonic gas distally); (c) paralytic ileus; (d) indistinct outline of kidney and psoas muscle; or (e) diffuse haziness in the presence of ascites (67).

An upper gastrointestinal series may reveal displacement of the stomach from a retroperitoneal mass, enlargement and widening of the duodenal C loop, effacement of the medial duodenal wall, and delayed gastric emptying.

Chest radiography may reveal pleural effusion, platelike atelectasis, pneumonia, pulmonary edema, and elevation of one or both hemidiaphragms.

Abdominal ultrasonography (68) in patients with acute pancreatitis is technically unsatisfactory in approximately one-third of patients. This may be due to an associated ileus or excessive bowel gas. When visualized by ultrasonography, the changes seen in the pancreas are nonspecific (for example, enlargement). This examination is most useful to diagnose a pseudocyst and provide localization for percutaneous drainage of such, or for the determination of cholelithiasis.

Computed tomography (CT) (69) is not limited by the presence of adipose tissue or the presence of bowel gas in its ability to visualize the pancreas. In severe pancreatitis, CT scanning has been shown to be normal in 70% of patients and abnormal in all patients with severe (hemorrhagic) pancreatitis. It is most useful in diagnosing the complications (e.g., abscesses, phlegmon, pseudocyst) of pancreatitis.

The use of ERCP in the diagnosis of pancreatitis is discussed above (see "Idiopathic Pancreatitis").

Differential Diagnosis. Multiple abdominal and thoracic diseases may mimic acute pancreatitis. These entities include perforated duodenal ulcer; choledocholithiasis; inferior-wall myocardial infarction; renal colic; mesenteric vascular occlusion; small-bowel obstruction; dissecting aortic aneurysm and pneumonia. Shock or coma of uncertain etiology should always suggest the possibility of acute painless pancreatitis, which occurs in less than 2% of patients, but which has a grave prognosis (58).

Therapy. Generally, the aim of therapy in acute pancreatitis is to "place the pancreas at rest" and thus reduce pancreatic enzyme synthesis and secretion. Although this is theoretically desirable and an altruistic objective, it is rarely accomplished, because once intrapancreatic enzyme activation is initiated, it is self-perpetuating. Clinically, the prescription for the patient is nothing by mouth until symptoms resolve. Nasogastric suction is useful for the patient with an ileus or nausea with vomiting. It has limited value in patients with mild or moderate pancreatitis, not complicated by an ileus (70).

In 75%–90% of patients, acute pancreatitis will resolve spontaneously with supportive care alone (71, 72)—including restoration of intravascular volume, because the basic pathology is a retroperitoneal burn, and relief of pain (meperidine hydrochloride intramuscularly, in preference to morphine; the former induces less spasm of the sphincter of Oddi). In addition, there must be prompt recognition and vigorous treatment of complications such as cholangitis, hypoxemia, renal failure, hypocalcemia, hyperglycemia, and bleeding. If hypotension persists despite adequate volume replacement, intravenous pressor agents are required. Other measures that have been used include peritoneal lavage to attempt to remove vasoactive substances from the peritoneal cavity (73); in initial noncontrolled studies and in a recent controlled study, this procedure did not reduce overall mortality.

In the setting of an uncertain diagnosis, deteriorating clinical condition, biliary pancreatitis, pancreatic abscess, or colonic necrosis, surgical intervention may be indicated (74). In patients with biliary pancreatitis, cholecystectomy is usually performed within 72 hr of admission. Alternatively, endoscopic sphincterotomy may be performed in acute biliary pancreatitis (75). This results in rapid alleviation of symptoms and rapid return of laboratory values to normal. In patients with severe necrotizing pancreatitis, emergent laparotomy for drainage, with or without peritoneal lavage (76), and near-total to total pancreatic resection for treatment of necrotic pancreas have been advocated (77). Unfortunately, these therapeutic options have not significantly increased survival.

Prognosis. Ranson et al have identified criteria that predict the presence of severe disease with an associated increased mortality in patients with acute pancreatitis (78) (Table 3). These criteria are divided into those identified on admission, or within the first 24 hr following admission, and those identified within the initial 48 hr of admission. The presence of three or more of these criteria either on admission or within 48 hr entails a prediction of 62% mortality; patients with less than three of these criteria have a predicted mortality of 3%. These criteria have recently been somewhat simplified by Bank et al (79). Basically, they found that in acute or acute relapsing pancreatitis, if one or more organ systems in addition to the pancreas were affected, the mortality was 56%; if no other organ systems were affected, mortality was only 2%.

TABLE 3

Ranson Criteria for Severe Acute Pancreatitis

At admission	Within first 48 hr
Age > 55 years	Hematocrit drop > 10%
WBC > 16,000/mm^3	BUN increases > 5 mg %
Blood glucose > 200 mg %	Serum calcium < 8.0 mg/dl
Serum LDH > 350 IU/liter (nl: up to 225)	Arterial pO_2 < 60 mm Hg
SGOT > 250 S.F.U (nl: up to 40)	Estimated fluid sequestration > 6 liters

Complications: Pseudocysts. Pseudocysts occur in approximately 25% of cases of acute pancreatitis. Approximately 85% resolve with no intervention within the first few weeks after diagnosis (80). They should be suspected clinically in the setting of the persistence of pain, fever, nausea, vomiting, or elevated serum amylase for more than one week. Ultrasonography or CT scanning is the procedure of choice for confirming the diagnosis, although the latter may prove to be more accurate. Potential complications directly related to pseudocysts include: (a) spontaneous rupture into the peritoneum or pleural cavity or into a hollow organ; (b) obstruction of contiguous organs, that is, common bile duct, stomach; and (c) erosion into the gastrointestinal tract, which results in bleeding. Since the natural history of the majority of pseudocysts is spontaneous resolution within 6 weeks, definitive therapy should not be instituted in the asymptomatic patient until this time. If the pseudocyst is greater than 4 cm in diameter and unchanged or increasing in size, percutaneous aspiration under ultrasound or CT guidance should be performed (81, 82). In one-third of cases, this modality alone will be successful in treating the cyst, and when it is combined with prolonged catheter drainage, its success rate increases significantly. If unsuccessful, internal drainage with cystogastrostomy or Roux-en-Y drainage is the procedure of choice.

In the presence of temperature greater than 101°F, increasing pain, palpable abdominal mass, leukocytosis, tachycardia, chills, hypotension, or positive blood cultures, the diagnosis of pancreatic abscess or infected pseudocyst must be considered. It is usually not clinically possible to distinguish a sterile pseudocyst from an abscess when temperature elevation and leukocytosis are present. Therefore, to differentiate a sterile from an infected pseudocyst or abscess, percutaneous aspiration is mandatory (82, 83). If bacteria with leukocytes are present on aspiration or cultures are positive, catheter drainage combined with appropriate antibiotic therapy must be instituted for the infected pseudocyst. If percutaneous catheter drainage is unsuccessful in the infected pseudocyst, surgical drainage is needed. Surgical drainage is also needed for adequate drainage of a pancreatic abscess.

Complications: Bleeding. Gastrointestinal bleeding during an episode of acute pancreatitis may be specifically related to the pancreatitis or to usual causes. Those related specifically to the pancreas include antral and/or duodenal erosions from contiguous pancreatic inflammation and "pancreaticosuccinus"—bleeding originating in the pancreas and flowing out through the ampula of Vater. Pancreaticosuccinus bleeding can also originate from erosion of a pseudocyst or abscess into the gastroduodenal or splenic arteries. Gastrointestinal bleeding can be secondary to fistula formation from a pseudocyst or abscess into the lumen of the stomach, duodenum, colon, esophagus, or common bile duct. Prompt, aggressive diagnosis and treatment must be instituted.

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Chronic Pancreatitis: Update 1986

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The Marseilles delegates have defined chronic pancreatitis as a chronic pain syndrome characterized by progressive and permanent functional and morphologic damage to the pancreas. Many patients with chronic pancreatitis, however, may have acute exacerbations.

Incidence. A retrospective analysis in Minnesota yielded an incidence of 3.5 per 100,000 population versus 10 per 100,000 of the population who had acute pancreatitis.

Pathogenesis. The exact pathogenetic mechanisms involved in chronic pancreatitis are not known. It has long been speculated that pancreatic secretory proteins precipitate in the pancreatic duct, where they calcify and cause obstruction (1). Obstruction then causes chronic inflammation with persistent and progressive destruction of acinar and islet cell tissue (2).

Microscopically, these changes range from acute interstitial edema with a leukocytic infiltrate, to foci of necrosis and abscess formation. Pursuant to the chronicity of this disorder, there is progressive destruction of pancreatic tissue with formation of fibrous tissue, scarring, and replacement of both islet cell and acinar elements. Periductal fibrosis leads to stricture formation. Grossly, the pancreas is shrunken and nodular, with scattered areas of parenchymal calcification.

Precipitating Factors. Unfortunately, the exact etiology of chronic pancreatitis remains uncertain, but several associated conditions have been identified.

In the United States approximately 70%–80% of patients with chronic pancreatitis are chronic alcoholics. At autopsy, it was found that 45% of alcoholics who had no symptoms of pancreatitis during their lives had the morphologic changes of chronic pancreatitis (3). This incidence is 40–50 times greater than in nondrinkers. Thus, since many, if not most, alcoholics have suffered permanent functional and morphologic changes of chronic pancreatitis by the time they suffer their first episode of acute alcoholic pancreatitis, they may be considered to have chronic pancreatic disease (4). Serial endoscopic retrograde pancreatography in patients with chronic alcohol-induced pancreatitis has shown that pancreatic duct abnormalities seen early in the course of the disease often progress, despite abstinence from alcohol (5).

Chronic pancreatitis is rarely believed to occur even after recurrent episodes of gallstone-associated acute pancreatitis. This is supported by the observation that when alcoholism and gallstones exist together in patients with chronic pancreatitis, the course of the disease is compatible with that of alcohol-induced chronic pancreatitis.

Hypercalcemia secondary to hyperparathyroidism or other conditions may theoretically be present with acute pancreatitis, which may progress to irreversible changes of chronic pancreatitis.

Hyperlipoproteinemia types I, IV, or V may lead to acute as well as chronic disease. Acute pancreatitis usually occurs when serum triglyceride levels are above 1,000 mg/100 ml and recurrent eipsodes are decreased with reduction of serum triglycerides to levels below 500 mg/ml (6).

Blunt abdominal trauma may cause pancreatic duct injury. There may be imcomplete healing or stenosis secondary to this ductal injury. These obstructive changes in the duct may lead to chronic pancreatitis.

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The initial description of hereditary pancreatitis was in five kindred who developed recurrent attacks of abdominal pain beginning in early childhood (7). Of the 38 patients studied, 21 developed one or more of the sequelae of chronic pancreatitis. Unfortunately, a thirtyfold increased incidence of pancreatic carcinoma has been found to occur in this syndrome.

Clinical Manifestations. The vast majority of patients have a history of abdominal pain, although this may be absent in 10% of cases (8). Typically, the patient is a persistent drinker who has exocrine insufficiency, and the clinical picture is dominated by intermittent or continuous pain. This picture evolves into the cardinal clinical and laboratory features that are due to impaired exocrine and endocrine pancreatic function. These features include weight loss, steatorrhea, diabetes mellitus, and undigested muscle fibers in the stool with increased stool nitrogen.

Although abdominal pain dominates the clinical picture in chronic alcoholic pancreatitis, it has become clear that the natural history of the disease is pain reduction. Eighty-five percent of patients (120 of 141) with calcific alcohol-induced chronic pancreatitis obtained lasting pain relief within a median time of 4.5 years from the onset of the disease (9). This study suggests that pain in chronic pancreatitis is directly related to pancreatic secretory capacity and that, with progressive glandular destruction and decreased secretory capacity below a critical level, the substrate for pain disappears.

Tests of Pancreatic Function. The diagnosis of chronic pancreatitis is usually clinically obvious. However, when the diagnosis is in doubt, pancreatic exocrine function testing is useful.

Intravenous injection of secretin followed by aspiration and analysis of duodenal contents is the most reliable test to determine pancreatic exocrine function (10). The total output of bicarbonate is the best correlate with pancreatic function.

Other means of assessing pancreatic exocrine function have been employed. Tests for intestinal trypsin, stool chymotrypsin, and intestinal chymotrypsin have all been developed, but none are as sensitive as the secretin test in detecting mild pancreatic insufficiency. The para-aminobenzoic acid (PABA) test provides an indirect measurement of intestinal chymotrypsin activity (11). An oral dose of *N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid is administered, which is cleaved by chymotrypsin. Greater than 60% of the cleaved PABA is absorbed in the intestine, conjugated in the liver, and then excreted in the urine. Since chymotrypsin activity is a reflection of pancreatic function, low levels of PABA in the urine are compatible with pancreatic insufficiency.

This test is quite reliable in the presence of pancreatic insufficiency associated with steatorrhea, but there is a high percentage of false negatives when only mild to moderate pancreatic insufficiency is present. This test is not as sensitive as the secretin test for detection of mild pancreatic insufficiency. False positive results occur in the presence of diffuse intestinal disease, chronic liver disease, and renal insufficiency.

Malabsorption due to pancreatic insufficiency is determined by direct measurement of fecal fat excretion. The patient is placed on a standard 100 gm/day fat diet, and levels of fat excretion greater than 5–7 gm/day are considered abnormal. Of interest is the fact that the pancreas has enormous functional reserve. Fat and protein malabsorption ensues only after 90% of secretory capability is lost (12).

Radiologic Abnormalities. Abdominal x rays may show diffuse pancreatic calcifications or intraductal lithiasis. Long-bone films reveal medullary infarcts or aseptic necrosis of humeral or femoral heads resulting from necrosis of medullary fat.

Hypotonic duodenography may show effacement of the medial descending duodenum, a prominent ampulla of Vater, mucosal spiculation, or thumbprinting in the duodenal bulb. Upper gastrointestinal series may reveal indirect evidence of pancreatic enlargement with displacement medially of the gastric antrum and widening of the duodenal loop.

Endoscopic retrograde pancreatography demonstrates abnormalities of the main and secondary ducts including ductal dilatation, scattered areas of narrowing, cysts, and ductal calculi.

Ultrasonography and computerized tomography scanning may reveal a dilated main pancreatic duct, pancreaticolithiasis, and parenchymal calcifications, which were not visualized on plain film of the abdomen. They are most useful for revealing pseudocysts and abscesses.

Differential Diagnosis. Generally, the diagnosis of chronic pancreatitis is not challenging. The history of alcoholism combined with weight loss, diabetes, or evidence of malabsorption readily reveals the diagnosis. However, pancreatic carcinoma may sometimes prove difficult to differentiate; cytologic aspirates or wedge biopsies may be needed to confirm malignancy. Patients with celiac sprue may also have abnormal responses to secretin testing. Biopsy of jejunum will confirm the diagnosis of sprue.

Treatment. Since the majority of patients with chronic pancreatitis have the complex of abdominal pain and evidence of pancreatic insufficiency, the main steps of therapy are pain relief and pancreatic enzyme replacement.

Classically, pain relief was achieved through use of meperidine (Demerol). Morphine was avoided because of its theoretical constricting effect on the sphincter of Oddi. Most recently, megadoses of oral pancreatic enzymes have been utilized for pain relief. Toskes and colleagues treated patients suffering from chronic abdominal pain secondary to chronic pancreatitis with high-dose (32 tablets daily) Ilozyme (13). Most patients with mild to moderate, but not severe, pancreatic insufficiency had pain reduction. Therefore, it is felt that high-dose pancreatic extract should be the initial treatment for the majority of patients with recurrent abdominal pain and chronic pancreatitis.

Exocrine insufficiency is reversed with administration of exogenous enzymes. These are administered prior to meal consumption. A high-potency non-enteric-coated product should be utilized. Occasionally, large supplements of pancreatic enzyme may still prove ineffective. This may be due to inactivation of the exogenous enzymes by gastric acid. An H_2 receptor blocker, such as cimetidine, before meals may prove useful in decreasing acid degradation of the pancreatic enzymes (14). Whether this will improve the pain-relief effect is unknown. Histamine antagonists are best utilized in patients who have pancreatic exocrine insufficiency that is unresponsive to the usual doses of enzyme replacement.

Three other measures that have been utilized to control pain in patients with chronic pancreatitis are nerve blocks, endoscopic procedures, and surgical intervention.

Celiac plexus block has been used in patients with chronic pancreatitis for pain relief. Although 12 of 23 treated patients obtained total relief of pain, and 6 other patients obtained partial pain abatement, the benefits lasted only for a mean of two months (15). Thus short-term relief can be obtained, but long-term effects are unfortunately lacking.

Endoscopic retrograde pancreatography has been used to aspirate and remove protein plugs for pain relief in patients with chronic pancreatitis (16). This modality achieved rapid pain relief in 17 of 69 patients. This measure, because of plug recurrence, is considered only temporary. Endoscopic duct obstruction by installation of alcoholic prolamine solution in patients with chronic pancreatitis has also been attempted for pain relief (17). Of 21 patients treated with ductal occlusions, all but one were completely relieved of pain within one week. However, long-term results were not nearly as promising, and the authors concluded that ductal occlusion will probably not give long-lasting pain relief. Endoscopic pancreatic sphincterotomy has also been employed in the treatment of chronic pancreatitis. Fuji et al successfully performed pancreatic sphincterotomy in 10 of 13 patients with chronic pancreatitis, and improvement in pain occurred in 9 patients (18). Moreover, after pancreatic sphincterotomy, in 2 patients stones were removed from the main pancreatic duct using a basket via a peroral oblique viewing pancreatoscope. In addition, in 3 other patients following pancreatic sphincterotomy, a pancreatic endoprosthesis was placed endoscopically into the main pancreatic duct to improve pancreatic drainage. Long-term follow-up and success rates of such new technologies await further investigation.

Numerous surgical procedures have also been advocated for pain relief in chronic pancreatitis. Multiple procedures have been specifically designed to decompress the pancreatic duct based on the underlying belief that pain is perpetuated by duct obstruction. Operative pancreatic duct drainage has been reported by Morrow and associates to afford pain relief in 80% of patients (19). Lateral pancreaticojejunostomy, in which the entire length of the duct is opened and joined to the jejunum, has been found to afford pain relief in more than 70% of patients (20). Pancreaticogastrostomy, in which the pancreatic duct is directly anastomosed to gastric mucosa, has recently been proposed as an alternative to lateral pancreaticojejunostomy (21). The advantage of pancreaticogastrostomy is purported to be less leakage and disruption of the anastomosis, but this is still under investigation. The effect of lateral pancreaticojejunostomy on fat absorption in patients with chronic pancreatitis remains to be further delineated (22).

Patients in whom ductal drainage fails to relieve pain and who have severe pancreatic fibrosis along with small or normal-sized major pancreatic ducts may be considered for pancreatic resection (17). The results of 95% resection seemed encouraging initially, but long-term problems, including high incidences of pancreatic exocrine and endocrine insufficiency leading to death, have been found (23, 24). Islet cell autotransplantation, to alleviate the diabetes induced by pancreatectomy, is still an investigative modality (25).

Complications. The complications of pseudocysts and abscesses associated with pancreatitis have been discussed in the companion article on acute pancreatitis.

Common bile duct stricture or stenosis secondary to chronic pancreatitis has been increasingly recognized. It occurs in approximately 10%–15% of patients with chronic pancreatitis. It should be suspected in the presence of any one of the following: persistently elevated alkaline phosphatase, elevated serum bilirubin, liver histology consistent with extrahepatic obstruction or secondary biliary cirrhosis, or dilatation of the common bile duct (26). Petrozza et al have found that more than four weeks of elevation of serum alkaline phsophatase of more than 2 times normal can be reliably used as a screening test for detection of common bile duct stenosis in patients with alcoholic pancreatitis (27). When bile duct stenosis is suspected, demonstration of the anatomy of the common bile duct with either endoscopic retrograde cholangiopancreatography or percutaneous transhepatic cholangiography is mandatory. The sequelae of such common bile duct strictures are cholangitis, biliary stone formation secondary to biliary cirrhosis, and pyogenic liver abscess (28, 29).

Duodenal ulcers have been found more frequently among patients with chronic pancreatitis than among those with normal pancreatic function (30). The postulated mechanism is impaired pancreatic secretion of neutralizing bicarbonate, resulting in excessive luminal acidity in the proximal duodenum.

Anatomically, the inflamed pancreas may come into contiguity with the splenic vein, resulting in splenic vein thrombosis and, rarely, extrahepatic portal hypertension. When this occurs, the spleen may rapidly enlarge. Thrombosis of the vein leads to intrasplenic venous hypertension, creating collateral formation via the short gastric vessels. This leads to gastric varices, which may rupture and cause upper gastrointestinal hemorrhage. The diagnosis is made by a high index of suspicion and by selective splenic artery angiography or splenoportography. If, indeed, these radiologic studies reveal that the splenic vein is occluded, that the portal vein is patent, and that the varices represent venous collaterals arising from the spleen, the curative treatment is splenectomy. Endoscopy may reveal gastric varices.

Prognosis and Natural History. The natural history and prognosis of chronic pancreatitis are poorly defined. In 1970, Ammann and colleagues in Switzerland reported a 20-year prospective study of 245 patients with chronic pancreatitis, including 163 with alcoholic relapsing pancreatitis, 145 of whom had calcific pancreatitis (9). The patients were studied at approximately yearly intervals by evaluation of pain, pancreatic function, calcification, pancreatic surgery, and survival. The group with alcoholic relapsing pancreatitis was observed for a mean period of 10.4 years. In this group, 85% experienced lasting pain relief within a median time of 4.5 years from onset. As the duration of the disease increased, a gradual increase of pancreatic calcifications and pancreatic dysfunction was observed, and pain relief was correlated with increasing disease duration. Of the group with alcoholic relapsing pancreatitis, 53% required no pancreatic surgery. The other 47% with recurrent or persistent severe pain, mainly due to pseudocysts, underwent either a cyst drainage procedure, papillotomy, distal pancreatectomy, or Wirsungojejunostomy. The proportion of patients experiencing lasting pain relief was similar in the operated-on and nonoperated-on groups of patients. In both groups, lasting pain relief correlated with disease duration and was associated with marked pancreatic dysfunction. The 50% survival time in alcoholic chronic pancreatitis, with or without pancreatic surgery, was 20–24 years after onset of disease. This was markedly shorter (by approximately 20%) than in nonalcoholic pancreatitis. Of the total 245 patients studied, 86 died. Approximately 20% of deaths were related to pancreatitis and its complications. The extrapancreatic causes of death were malignancies, cardiovascular disease and sudden death, severe infections, postoperative nonpancreatic surgery, cirrhosis/hepatitis, and gastrointestinal bleeding.

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THE MOUNT SINAI JOURNAL OF MEDICINE

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THE MOUNT SINAI JOURNAL OF MEDICINE AWARDS 1985/86

Kant Yuan-Kai Li

Student Prize

The 1985/86 Student Prize for the best student paper published annually in *The Mount Sinai Journal* has been awarded to Kant Yuan-Kai Lin, MD, for "Acute Acalculous Cholecystitis: A Limited Review of the Literature" (vol. 53, no. 4), written when the author was a fourth-year medical student at Mount Sinai School of Medicine (CUNY). Dr. Lin is currently completing his second year of surgical training at the Hospital of the University of Pennsylvania. This and the other *Journal* prizes were presented at the School of Medicine graduation awards ceremony May 30, 1986.

Steven A. Kapla

Ralph Colp Prize

The 1985/86 Ralph Colp Prize for the best surgical paper published in *The Mount Sinai Journal* during the academic year went to Steven A. Kaplan, MD, for his article "Orthotopic, Segmental Small-Bowel Transplantation in the Rat" (vol. 53, no. 2). His co-authors were Daniel Racelis, BS, Giorgio P. Martinelli, PhD, Harry Schanzer, MD, and Arthur H. Aufses, Jr., MD. Dr. Kaplan, who graduated summa cum laude from Brooklyn College with a BS in biochemistry, is now senior resident in urology at Columbia-Presbyterian Medical Center. Dr. Kaplan plans an academic career in urologic oncology and neurology.

Ezra M. Greenspan

Globus Award

The 1985/86 Globus Award for the best clinical paper published annually in *The Mount Sinai Journal* went to Ezra M. Greenspan, MD, for his paper "Prior Tuberculosis in Long-Term Survivors of Breast and Ovarian Cancer from a New York Oncology Practice (1954–1974)" (vol. 52, no. 6). Dr. Greenspan, clinical professor of medicine (oncology) at the Mount Sinai School of Medicine and founder and medical director of the Chemotherapy Foundation, NYC, demonstrated in pioneering research first published in *The Mount Sinai Journal* in 1962 and 1963 that ovarian and breast cancer could be more effectively treated by combinations of drugs than by the single drugs then in use. Rudo S. Globus, PhD, presented the award memorializing his father, Joseph, founder and first editor of the *Journal.*

Carol Victor-Kobrin

Francisco A. Bonilla

Mount Sinai Journal Award

Carol Victor-Kobrin, PhD, Francisco A. Bonilla, BA, and Constantin A. Bona, MD, PhD, were the recipients of the 1985/86 Mount Sinai Journal Award for the best research or experimental paper published in the award year for their paper "An Immunochemical and Molecular Analysis of Monoclonal Antibodies Bearing the A48 Regulatory Idiotype" (vol. 53, no. 3). Dr. Bona declined the award in favor of his junior co-authors. Dr. Victor-Kobrin's interest in the role of idiotypy in immune regulation grew out of postdoctoral research carried out in Dr. Bona's microbiology laboratory at Mount Sinai Medical Center in New York. Mr. Bonilla, an MD-PhD candidate in microbiology in the Medical Scientist Training Program, wishes to extend his warm congratulations to his former classmates, the graduating Mount Sinai School of Medicine class of 1986.

Pulmonary, Vascular, and Pediatric Papers

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Nonneoplastic Asbestos-Induced Disease

ANDREW CHURG, M.D.

Asbestos induces a wide variety of both neoplastic and nonneoplastic diseases of the lungs, pleural cavity, and peritoneal cavity. This article considers some current areas of pathologic and clinical controversy related mostly to nonneoplastic conditions; the various asbestos-induced tumors are examined in detail in the accompanying paper by Dr. McCaughey.

Mineralogically, asbestos is not a single substance, but rather a variety of silicate minerals which are natural fibers, all referred to by the same term. These fibers are of commercial importance because they are, in general, highly resistant to heat and to chemical attack, and because they have extremely high tensile strength and, in some instances, high spinability. For these reasons asbestos is used in a wide variety of commercial products, of which the majority are construction and insulation materials. Asbestos is also incorporated into such items as floor tiles, ceiling insulation tiles, friction materials such as automotive brake linings, fireproof textiles, and a variety of other products. It has been estimated that at one time more than 3,000 products contained asbestos (1).

Most asbestos exposure is occupational in origin and is limited to miners and millers of asbestos; construction workers of all types, including plumbers, electricians, carpenters, welders, and masons; workers in factories manufacturing specific asbestos products, such as textiles; laggers, pipefitters, and insulators of all types; and even automotive-brake-manufacturing workers and mechanics (2, 3). However, the widespread use of asbestos in numerous products, as well as the previous practice of spraying asbestos insulation in open construction sites, has led to considerable contamination of the atmosphere. For this reason the whole population of North America is exposed to asbestos in very low concentrations (4). Low-level exposure also occurs in public buildings, such as schools, in which asbestos insulation has been used in ceilings or walls (5). Although the effects of occupational-level exposure to asbestos are generally well known, the effects of this low-level exposure to asbestos are disputed. Theoretic calculations have been performed which suggest that very low incidences of tumors such as mesothelioma might result from such exposures. These calculations are all extrapolations from data generated in the occupational setting, and their validity has never been proven; even if the numbers generated are correct, it should be appreciated that this incidence of potential disease is, as pointed out by a recent Royal Commission report (6), well below the dangers encountered in such activities as driving to work.

Asbestos Types and Their Biologic Impact

As noted above, asbestos is not a specific mineral. There are, instead, six different minerals which generally are regarded as asbestos.

Types. Some 90%–95% of current asbestos production and usage is chrysotile, or white asbestos, most of which comes from Quebec. The remaining types of asbestos fiber are members of a large mineral group called the amphiboles. Of

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Presented at a scientific meeting in honor of Drs. Jacob Churg and Lotte Strauss, "Selected Topics in Pulmonary, Vascular, and Pediatric Diseases," at the New York Academy of Medicine, June 14, 1985.

Supported by grants from the National Cancer Institute of Canada and the Medical Research Council of Canada.

these, amosite and crocidolite, both mined in South Africa, are used commercially to make asbestos products. Tremolite, actinolite, and anthophyllite asbestos are, as a rule, not mined as commercial products, but they do occur as contaminants of other minerals, particularly chrysotile ore.

Impact. These distinctions in mineral types are of biologic importance. Chrysotile tends to fragment and to dissolve in lung tissue, so that chrysotile tends to disappear from lung with time. Long chrysotile fibers are also curved and hence tend to impact fairly proximally in the airways. By contrast, the amphibole minerals are extremely stable in lung tissue and appear to persist with time. Their straight shape also probably leads to more distal deposition than is true for many chrysotile fibers (7).

The different types of asbestos minerals appear to be associated with differences in disease incidence, particularly in the area of mesothelioma (see accompanying article). The situation for asbestosis and benign pleural disease is uncertain, and at this point the most accurate statement is that all the types of asbestos minerals can cause either pleural or parenchymal fibrosis.

Bare Fibers and Ferruginous Bodies. Asbestos is inhaled as the bare mineral fiber. However, in the lung the asbestos is found in two forms: the bare fiber, and the asbestos body, a fiber of asbestos to which lung macrophages have added a coating of an iron protein. Suzuki and J. Churg were the first to examine this process in detail and to show that the coating was closely related to hemosiderin (8). The asbestos body coat has a distinctive golden color and is readily visible in the light microscope. By contrast, asbestos fibers are for the most part invisible in the light microscope.

The nature of asbestos bodies has been a matter of dispute (9). They originally were found in asbestotics, and were initially called "asbestosis bodies," until it was realized that they could be observed in the lungs of any worker exposed to asbestos, even if asbestosis was not present. However, in 1963, Thompson et al (10) reported that identical structures could be found in the lungs of routine-autopsy subjects in Cape Town, despite the fact that these patients had no history of occupational exposure. This observation has subsequently been confirmed in a large number of studies from South Africa, Europe, and North America, and at this point it is clear that asbestos bodies can be found in the lungs of virtually everyone in the population (9). In most individuals, these structures appear to reflect atmospheric contamination by asbestos fibers.

The notion that the bodies found in the lungs of the general population really were formed on asbestos was challenged by Gross and colleagues (11), who demonstrated that structures identical to asbestos bodies could be produced in animals with other fibrous minerals, for example, fiberglass or aluminum silicate. For this reason he suggested that such bodies be called "ferruginous" and claimed that, in the general population, they were not formed on asbestos. We have examined this issue by analyzing more than 600 bodies from a variety of members of the general population and have shown that virtually all are formed on amphibole asbestos (9). We have also examined bodies from workers with different types of occupational exposure and demonstrated that bodies form on chrysotile asbestos when there is sufficient exposure (12). As a rule, therefore, any structure that looks like an asbestos body, that is, has a golden coat and a fiber core that is colorless, transparent, and usually straight, can be assumed to be formed on asbestos.

Other types of ferruginous bodies do occur in humans. For the most part, these have cores that are either platelike or of different colors and are readily separated from true asbestos bodies. The one exception to this rule are bodies formed on the fibrous mineral erionite, an asbestos-sized mineral found in the soil in localized areas in Turkey. This mineral forms bodies that are morphologically identical to asbestos bodies, but to the best of our knowledge the phenomenon occurs only in Turkey (13).

Exposure vs Disease. Not only are asbestos bodies found in the lungs of everyone in the population, but uncoated fibers are also present, and in considerably larger numbers (14). For the most part these asbestos fibers consist of short pieces of chrysotile (less than 5 microns long), with relatively small amounts of amosite or crocidolite. Because very short fibers of asbestos do not form asbestos bodies readily, most of the asbestos bodies in the population are formed on the amphiboles. It appears at this point that the background exposure does not cause any known disease; however, if one wishes to perform mineralogic analysis to demonstrate that asbestos exposure of some unusual degree has occurred in a specific case, it is necessary to know what background level of fibers is to be expected from general atmospheric pollution before abnormal exposure can be documented.

Even if unusually high levels of asbestos fibers or bodies are found in the lung, what they indicate is exposure, not disease. This observation is also true of asbestos bodies.

Benign Pleural Disease

Asbestos causes a variety of benign diseases of the pleura. These may for convenience be separated into pleural pseudotumors, pleural effusions, pleural fibrosis, and pleural plaques, although the latter two often overlap.

Pseudotumors. Pleural pseudotumors, or so-called rounded atelectasis, are lesions that appear as pleural-based mass infiltrates on chest radiograph or computerized tomography scan. Typically, at thoracotomy the surgeon will find scarring of the pleura with infolding of the lung; as the dissection proceeds, the lung suddenly opens up and the mass lesion disappears. Histologically, lung from such pseudotumors shows only a small amount of pleural and subpleural scarring. As a rule pseudotumors cause few or no clinical symptoms, but are often mistaken for malignancies clinically.

Effusions. Asbestos-induced pleural effusions were first described by Gaensler and Kaplan (15) and described in more detail by Epler et al (16). These effusions are usually recurrent, commonly subclinical, and frequently hemorrhagic, raising the question of mesothelioma. Histologically, the pleura reveals nonspecific chronic inflammation and fibrosis. What is particularly important is assuring that the effusion is not secondary to a malignancy; as defined by Gaensler and Kaplan, idiopathic asbestos effusion is an exclusionary diagnosis which can only be realistically considered if no tumor has appeared within 3 years. As a rule, no therapy is required for asbestos effusions, but occasionally there is sufficient pain or respiratory compromise to necessitate pleurodesis.

Asbestos effusions as a rule appear within the first ten years after onset of exposure to asbestos, although isolated effusions may also be seen later (16). It is thought that organization of asbestos effusions gives rise to pleural fibrosis (16), and perhaps to pleural plaques as well.

Fibrosis. Pleural fibrosis typically appears as a thickened pleura, often best seen as blunting of the costophrenic angles. The fibrosis may occur on either visceral or parietal pleura and is most obvious in the lower lung zones. There is some evidence that extensive pleural fibrosis may produce a minor degree of restrictive impairment; the rare patient with extremely severe fibrosis may have clinical symptoms due to encasement of the lungs (16–18).

Histologically, asbestos-induced pleural fibrosis is nonspecific. In active lesions associated with effusion, one may see a distinct organization: from fibrin on the pleural surface, to a layer of inflammatory cells, fibroblasts, and proliferating mesothelial cells, to an almost hyaline layer near the chest wall (Fig. 1). In inactive lesions only fairly acellular connective tissue is present.

Plaques. Pleural plaques are bilateral discrete, raised, collagenous lesions typically found over the posterior lower ribs and diaphragm (Fig. 2). Roentgenographic observation of calcified diaphragmatic plaques is considered a pathognomonic sign of asbestos exposure. Histologically, plaques are composed of virtually acellular collagen in a basketweave pattern (Fig. 3).

The origin of plaques is uncertain, and their pathogenesis is the subject of great speculation but little information (19). Presumably, they reflect an inflammatory process of some sort that occurs when asbestos reaches the pleural cavity (as, of course, must asbestos effusions and pleural fibrosis). Why some pleural reactions end as fibrosis and some as plaques is unclear; it has been suggested from experimental observations that this difference depends on the nature of the inflammatory response—macrophages versus neutrophils (20). A recent radiographic study by Epler and colleagues (16) points out that the roentgenographic distinction between plaques and pleural fibrosis is often difficult, a difficulty that can occur in pathologic studies as well.

Exposure vs Disease. Benign pleural disease should not be referred to as asbestosis (or pleural asbestosis), because most pleural disease has no functional consequences and serves only as a marker of exposure, whereas true asbestosis is a progressive condition associated with pulmonary impairment (see below).

Airways Disease: Asbestos vs Smoking

Whether asbestos affects the airways is a controversial issue. Large-scale studies of pulmonary function in asbestos workers have suggested that the frequency of an obstructive profile is higher than would be expected from cigarette smoking alone, and that, statistically, exposed smokers have more severe functional changes than nonexposed smokers. The presence of smoking obviously complicates these observa-

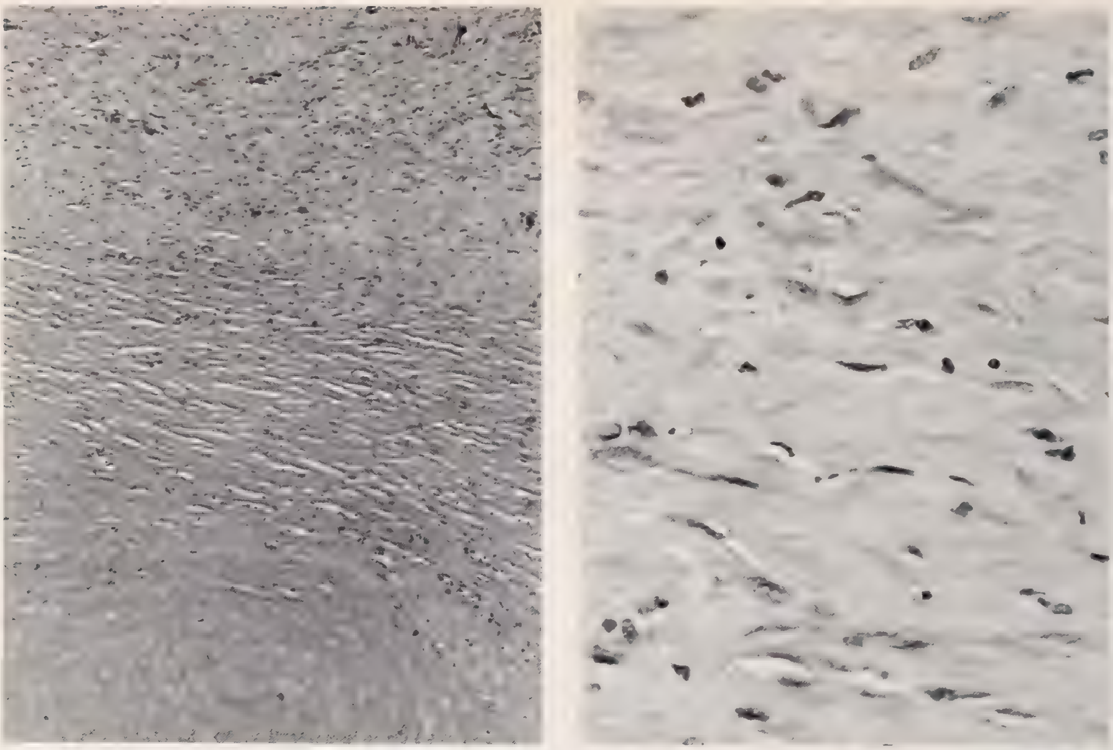


Fig. 1. Low- and high-power micrographs of an organizing asbestos effusion. Note distinct organization from cellular to acellular areas in low-power view (**left**) and proliferation of bland spindled cells in high-power view (**right**).

tions, but two recent studies have shown clearly that nonsmoking workers with asbestos exposure may have some degree of airflow obstruction, particularly as seen in the tests for small-airways dysfunction (21, 22). Whether asbestos ever produces clinically significant obstruction is a difficult issue, but it appears that in a cigarette smoker, obstruction must be primarily ascribed to cigarette smoke, and the role of asbestos, if any, is probably minor. Incidentally, there is no evidence whatsoever that asbestos exposure ever produces emphysema.

Morphologically, the functional changes appear to be associated with fibrosis of the small airways. This is most easily seen histologically in the respiratory bronchioles (Fig. 4), where the walls are greatly thickened and pigment deposition is often extensive. Careful studies have demonstrated that all of the noncartilagenous airways are abnormal and that these changes are widespread in workers with long-standing and high-level asbestos exposure (23). Morphologically similar lesions can be seen in workers exposed to other types of mineral dust, suggesting that all mineral dusts affect the small airways similarly (24).

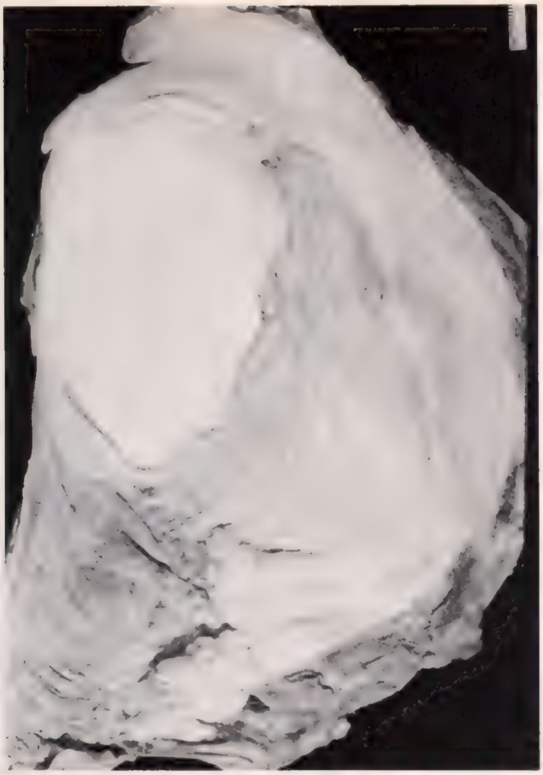
Asbestosis

Asbestosis is defined as diffuse fibrosis of the pulmonary parenchyma caused by asbestos exposure.

Diagnosis. The disease may be diagnosed purely on clinical grounds, including a trustworthy history of asbestos exposure, rales on auscultation, pulmonary function changes consistent with a restrictive disease, and a reticulonodular infiltrate most marked in the lower lung zones on chest roentgenograph. Not all these features are needed for diagnosis; see Murphy et al (25) for detailed criteria.

The diagnosis can also be made pathologically. Grossly, the typical case appears as lower-zone honeycombing and fibrosis which tends to spare the central portions of the lung. The pleura may have a cobblestone pattern. This gross appearance is nonspecific and can be seen in any type of diffuse interstitial fibrosis, particularly usual interstitial pneumonia. Diagnosis by microscope studies requires the presence of diffuse interstitial fibrosis and asbestos bodies seen in tissue section (Fig. 5).

Several caveats on pathologic diagnosis are



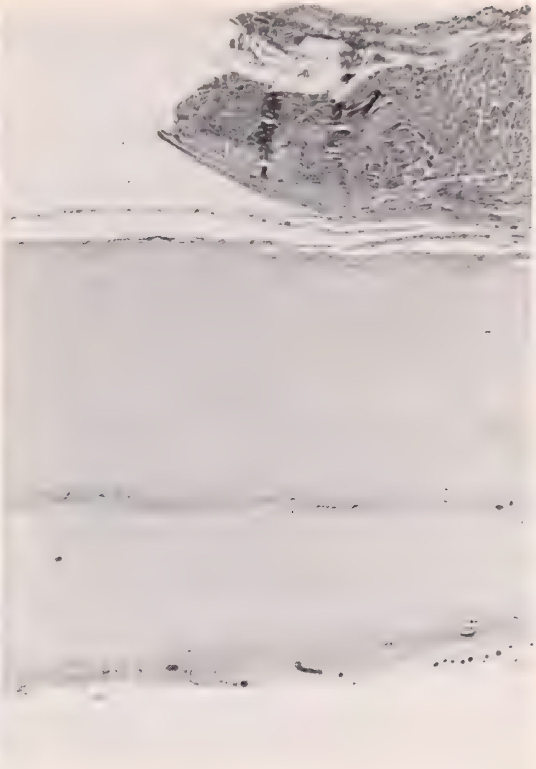


Fig. 2. Left Gross photograph of diaphragmatic pleural plaque.
Fig. 3. Right Micrograph of pleural plaque showing sharp demarcation and lack of cellular infiltrate. Chest wall muscle is at top of field.

in order. First, the pathologist must be sure that the fibrosing process is diffuse, since many kinds of nonspecific local scars, especially reactions around tumors, can produce fibrosis of the lung which in a small area mimics asbestosis if the patient has been exposed to asbestos and has asbestos bodies visible in tissue section. Second, if diffuse fibrosis is present, the finding of even one asbestos body serves to establish the diagnosis. Concern has been expressed that, because asbestos bodies from background exposure are present in the lungs of the general population, two or more bodies should be required for the diagnosis of asbestosis. However, asbestos bodies are so unusual in sections from the lungs of the general population, and interstitial fibrosis of any cause is such an uncommon disease, that it is unlikely the two events will occur together with any frequency. Roggli and Pratt (26) have calculated that one should not observe an asbestos body in more than one per hundred sections from a patient whose asbestos exposure is only atmospheric background, and then only if the sections are carefully and systematically searched using iron stains.

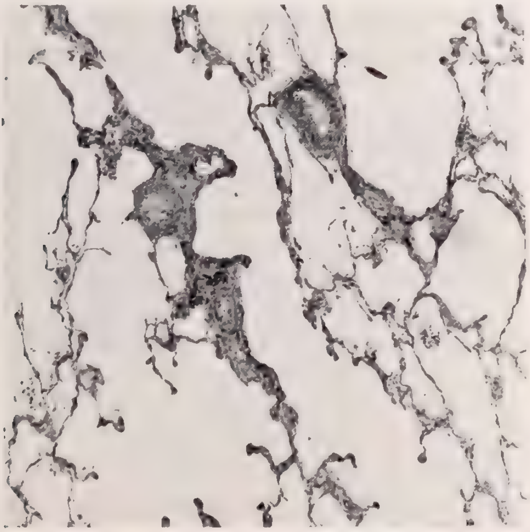


Fig. 4. Micrograph of asbestos airways disease. Note characteristic fibrous thickening and pigmentation of wall of respiratory bronchiole.

Some have claimed that the lesions described above as asbestos airway disease are in fact the earliest form of asbestosis. Others claim that the earlist lesions of asbestosis are a rather nebulous entity, "peribronchial fibrosis," which in some reports appears to mean the lesions in the airways I have described, and in others interstitial fibrosis around the airways (27). Recent observations in my laboratory suggest that interstitial fibrosis

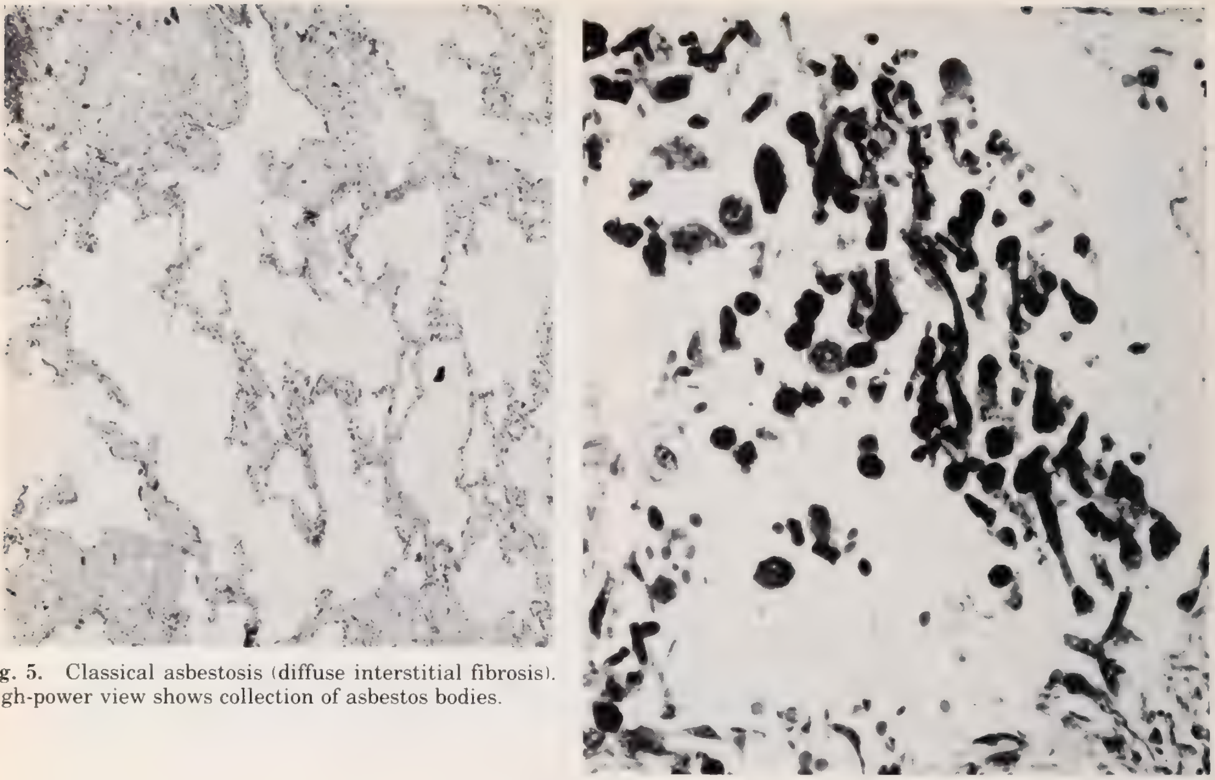


Fig. 5. Classical asbestosis (diffuse interstitial fibrosis). High-power view shows collection of asbestos bodies.

caused by asbestos may start around the airways where fibers penetrate the airway walls and gain access to the parenchyma, but the airway lesions appear to be a separate process (28). Certainly the airway lesions produce little clinical and definitely no radiographic disease, whereas ordinary parenchymal asbestosis is clinically and radiographically evident.

In most patients asbestosis can be diagnosed on clinical grounds. Biopsy should be reserved for cases in which either the history is uncertain, or the disease is clinically not typical of asbestosis. If a biopsy is to be performed, it is critical that open biopsy be used. Transbronchial biopsy is of little value in any kind of diffuse interstitial fibrosis, and careful studies have shown that prediction of the presence of diffuse interstitial fibrosis by transbronchial biopsy is more likely to be wrong than right (29). This conclusion applies to asbestosis as well. Moreover, the mere presence of asbestos bodies in any kind of biopsy indicates exposure only, not disease.

Course. Asbestosis as a rule appears to be a progressive disease. Death may result from respiratory failure, cor pulmonale, or the appearance of an asbestos-associated neoplasm. In series of asbestotics, carcinomas of the lung are frequently reported to account for 20% to 30% of deaths (30–32), and asbestosis, like other forms of pulmonary scarring, appears to predispose to the development of carcinoma. Mesothelioma is also seen in workers with asbestosis, but the association appears to be one of exposure rather than any specific sequence of pathologic events.

Association with Carcinoma. Considerable medical and legal interest focuses on the question of whether any specific types of carcinoma of the lung are associated with asbestos exposure, particularly with asbestosis. Kannerstein and J. Churg (33) were among the first to examine this question; they concluded that exactly the same cell types were seen in those with and without asbestos exposure. Whitwell et al (34) came to the opposite conclusion and declared that adenocarcinomas were most frequent in those with asbestosis. This latter assertion has been repeated many times, but the published data on the issue does not appear to support any overall difference in cell type based on exposure (35), and all types of lung cancer occur in those with asbestos exposure. Certainly one cannot use cell type in deciding whether a tumor should be ascribed to asbestos. When this question arises, I follow the rule adopted in the United Kingdom, which requires that asbestosis be present before a cigarette-smoking worker can receive compensation based on asbestos exposure for a carcinoma of the lung (36). However, other investigators use other rules for ascribing lung cancer to asbestos exposure in cigarette smokers (see article by McCaughey in this issue).

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Neoplastic Asbestos-Induced Disease

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Recognition of the carcinogenic effects of asbestos has evolved slowly over the past 50 years. Lung cancer first came under suspicion in the 1930s and 1940s, and by the 1950s it was well established in Europe as a frequent complication of asbestosis (1, 2). Strong evidence of an association between exposure to asbestos and mesothelioma appeared in 1960 (3), and subsequently the link has been confirmed by many studies. Finally, from 1964 onward evidence has been growing that asbestos may cause cancer in a number of other sites (Table). Cumulatively, these cancers of other sites add significantly to the mortality of asbestos workers (4).

In 1964 and 1965 several important papers concerning asbestos-related cancer came from the Mount Sinai group which included Drs. J. Churg, Selikoff, and Hammond (5–7). These reports described a striking excess of lung cancer and mesothelioma among American insulation workers and a probable excess of gastrointestinal cancer. It thus became obvious that asbestos-induced cancer was an important occupational disease in the United States and was not, as had sometimes been inferred, a disease mainly afflicting Europeans and South Africans. Subsequently, it was shown that many other categories of American asbestos workers were at risk and that paraoccupational neighborhood and domestic exposures to asbestos might also lead to cancer. Moreover, as awareness increased of the widespread contamination of the general environment by asbestos, it became apparent that there was at least a potential risk of asbestos-induced cancer for a large group of people in the general population.

Dr. Churg's special contribution to this unfolding story has come through the leadership and direction he has given to the evolution of objective criteria for the diagnosis of asbestos-related disease, especially mesothelioma. For many years he and his close friend and colleague, the late Dr. Milton Kannerstein, ran the U.S. Mesothelioma Panel and, in a number of important studies during that period, made us all more familiar with the characteristics of mesothelial tumors. The result has been that diagnosis of these controversial neoplasms is now much more objective, and related epidemiologic and clinical observations have become more secure.

Lung Cancer

Though it has been estimated that asbestos may cause some 5% of present-day lung cancers (8), the extent of the contribution of asbestos to the development of lung cancer in an individual patient may be difficult to quantify precisely, especially when the patient has been a cigarette smoker or the evidence of excessive exposure to asbestos is not clear cut. Initially asbestos was thought to cause lung cancer by multiplying the effects of tobacco smoke (9), but subsequently it was observed that asbestos caused lung cancer in nonsmokers (10–12). There is recent evidence that the relative risk of lung cancer in nonsmoking asbestos workers may be as high as or higher than in smoking asbestos workers (11, 12).

All commercial forms of asbestos have caused lung cancer, but some reports suggest that chrysotile, which accounts for more than 90% of the asbestos used in recent decades, is less potent than the two other widely used forms (amosite and crocidolite). However, in one setting (a chrysotile asbestos textile plant) the standard mortality ratio for lung cancer at different levels of exposure has been among the highest reported

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TABLE
Neoplastic Effects of Asbestos Exposure in Humans

Definite
- Lung
  - carcinoma
- Pleura and peritoneum
  - diffuse mesothelioma
- Gastrointestinal tract
  - carcinoma

Probable
- Ovary
  - carcinoma
- Larynx
  - carcinoma
- Pericardium and tunica vaginalis
  - diffuse mesothelioma

Probable/possible
- Reticuloendothelial system
  - malignant lymphoma
- Breast, kidney, and pancreas
  - carcinoma

for lung cancer in asbestos workers (13, 14). This may be due to the fact that textile processing produces a greater proportion of the form of asbestos fiber (long and thin) that is particularly carcinogenic. Experimental studies have also shown that fine chrysotile fiber is just as potent as other forms of asbestos in causing lung cancer (15).

The nature of the dose-response relationship between asbestos and lung cancer is particularly important in assessing the effects of low-level exposures. If, as occupational studies suggest, the relationship is linear or almost so (13, 14), very light exposures may proportionately increase the risk. No threshold level of asbestos exposure has been recognized below which no lung cancer risk exists. It is also apparent from dose-response projections that exposures much less than those required to produce asbestosis may cause lung cancer. Asbestos appears to act as a classic promoter in the development of lung cancer, though the pathogenetic mechanisms of asbestos-induced lung cancer are unclear (16). Because asbestos causes lung cancer in nonsmokers and experimental animals, it appears that the fiber may also have an initiating influence.

The frequency of the main cell types in asbestos-induced lung carcinoma appears to be about the same as in the general population. There is evidence, however, that asbestos lung cancers are more likely to be located in the lower lobes than lung cancers in the general population (17). The pathologist's assessment of whether a given lung cancer is related to asbestos exposure must obviously depend on the asbestos content of the lung and the occupational history. Because discrepancies in the two forms of evidence are sometimes marked, both must be taken into account.

Malignant Mesothelioma

Association with Asbestos. Recently, the long controversy over the status and the diagnosis of mesothelioma has been augmented because of certain aspects of its association with asbestos. Though this tumor has undoubtedly been increasing in frequency, it remains rare. Sixteen hundred cases are estimated to have occurred in the United States in 1980 (18). However, the tumor is probably still underdiagnosed in those not uncommon situations in which the behavior or histopathology is not typical or adequate material is not available.

When epidemiologic and lung-asbestos-burden studies are taken into account, it appears that asbestos has caused some 60% to 80% of pleural and peritoneal mesotheliomas. However, the reported extent of the association varies markedly in epidemiologic studies. Thus, in some reports over 80% of patients give an occupational exposure history, whereas in other reports the figure is less than 30% (see ref. 19 for references). One can only speculate on the extent to which these variations reflect varying occupational and environmental conditions in different parts of the world and the enthusiasm and knowledge of the history-taker. Only a small percentage of women with mesothelioma have been occupationally exposed (20, 21).

Quite possibly some people develop mesothelioma as a result of very low level nonoccupational exposure to asbestos. Supporting this possibility are extrapolations based on the dose-response relationship between asbestos and mesothelioma in occupational studies (22) and the fact that in both humans and experimental animals, mesotheliomas have been associated with brief exposures (15, 23). It is also known that nonasbestos forms of mineral fiber with similar physical characteristics (aspect ratio) to those of asbestos fibers may induce mesothelioma in experimental animals and humans. In humans, erionite, a fibrous form of zeolite not related to asbestos, has caused mesotheliomas in Turkey (24).

Much controversy in recent years centers on whether chrysotile asbestos induces mesothelioma. Most observers feel that it is less potent than amosite and crocidolite in this regard. Particularly notable is the fact that peritoneal mesotheliomas have rarely been observed in persons exposed to chrysotile. Experimentally, some forms of chrysotile have at least the same potential to induce pleural mesothelioma as other forms of asbestos. The role of chrysotile in initiating mesothelioma will have to be further evaluated in light of evidence that the tremolite form of asbestos, which is associated with chrysotile ore, could be a factor in the causation of pleural mesothelioma in chrysotile miners (25).

Other Factors. It is likely that factors or agents other than mineral fiber may be involved in the causation of some mesotheliomas (see ref. 19 for references). Thus, it has been suggested that radiation and chronic inflammation or scarring may have caused occasional mesotheliomas in humans. Genetic predisposition to cancer, as well as shared exposure to asbestos, may operate in the relatively frequent instances of familial occurrence of the neoplasm (26, 27). The list of agents that have caused mesothelioma in experimental animals is a long one (19) and further encourages a critical attitude on the etiology of mesothelioma.

Diagnosis. The pathologist can often diagnose mesothelioma in the early stages given experience, good material, and appropriate laboratory facilities. In several situations, however, considerable potential for misdiagnosis exists.

Cytologists are familiar with the fact that exuberant mesothelial hyperplasia can be readily confused with mesothelioma. Less well appreciated is the extent to which mesothelial hyperplasia may mimic tumor grossly and in biopsies (28). Focal mesothelial hyperplasias on occasion may be so pronounced as to form grossly visible nodules. Mild, and even moderate, cellular atypia may also be present, and—rarely—hyperplastic mesothelium may assume a papillary disposition. Occasionally, hyperplastic mesothelium may invade immediately underlying normal tissue, but necrosis and marked cytologic atypia do not occur in mesothelial proliferations proved to be benign on follow-up (28).

Dr. Churg has pointed out a small group of peritoneal mesotheliomas which run a very slow course and which often begin with what looks like hyperplasia (29). We have encountered occasional patients in whom marked mesothelial hyperplasia has been associated with chronic effusions and after several years of recurrent effusion, the cause of the mesothelial hyperplasia was not clear (28). In these situations the possibility that the hyperplasia is precancerous must be kept in mind.

Another difficult diagnostic problem in small serosal biopsies concerns differentiation of scar tissue from desmoplastic diffuse mesothelioma. This entity was delineated by Kannerstein and Churg some years ago (30). The tumor may contain extensive areas indistinguishable from banal scar tissue which may easily mislead the pathologist in small biopsies (30, 31). Further complicating the interpretation of fibrous serosal lesions is the fact that pleural fibrosis may sometimes be quite cellular and thus resemble fibrous tumor. The degree of nuclear atypia is usually the most critical factor in determining whether the tissue is benign or malignant. As in the case of mesothelial hyperplasia, necrosis favors malignancy (31). A well-developed tissue pattern—storiform or gyriform—also points to mesothelioma (28).

The most common diagnostic problem for the pathologist in biopsy material is differentiation of mesothelioma from metastatic carcinoma. When the tumor has a glandular or tubulopapillary pattern, the differential diagnosis is with adenocarcinoma. Positive staining for mucin with mucicarmine or periodic acid Schiff after diastase is usually considered to be decisive evidence of adenocarcinoma, although I have occasionally seen otherwise typical mesotheliomas with small numbers of tumor cells containing diastase-resistant PAS-positive granules or globules in their cytoplasm (32). Because a small proportion of adenocarcinomas arising in the lung and elsewhere do not secrete mucin, a negative reaction does not help significantly in distinguishing between mesothelioma and adenocarcinoma. Immunostaining for carcinoembryonic antigen is often helpful. Many adenocarcinomas immunostain strongly, but mesotheliomas do not, in our experience. However, mesotheliomas may occasionally react weakly. Though it has been claimed that positive immunostaining for human milk fat globule antigen distinguishes adenocarcinoma from mesothelioma (33), others have reported no discriminatory value (34).

Electron microscopy has been increasingly useful to us in distinguishing between mesothelioma and poorly differentiated carcinoma. In this situation the ultrastructural features (long slender microvilli, cytoplasmic cysternae, distribution of intermediate filaments) that help distinguish classical tubulopapillary mesothelioma and well-differentiated adenocarcinoma (35, 36) are often not helpful. In a recent study of poorly differentiated diffuse epithelial mesotheliomas, we have found that although the classical characteristics of well-differentiated neoplastic mesothelial cells are markedly modified, cytologic details remain which are useful in distinguishing mesothelioma from carcinoma. These include the presence of cytoplasmic processes lying parallel to the adjacent plasma membranes, abundant cytoplasm with a polar disposition of organelles, and disaggregation of nuclear chromatin (37).

Other Cancers

It seems certain that asbestos causes other cancers (Table), though the strength of the evidence supporting a relationship with individual types of cancer varies. The strongest evidence of an association is with gastrointestinal carcinomas (38) and ovarian cancer (39, 40). In view of the relationship with ovarian cancer, it is of interest that the common ovarian epithelial tumors are believed to be of mesothelial origin and that tumors histologically identical to ovarian serous carcinomas may arise from the peritoneum of females (41, 42). Because asbestos is rapidly transmitted through the wall of the intestine into the lymphatic circulation, there is obviously the potential to initiate cancer in virtually any part of the body.

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Hepatic and Renal Involvement in Malformation Syndromes

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Cystic abnormalities of both the liver and kidney occur in several groups of heritable syndromes, many known principally as examples of renal cystic disease (1). The same abnormalities can also occur principally as hepatic disease. The largest group of hepatic cystic diseases is classified under the broad heading of congenital hepatic fibrosis, occurring in association with autosomal recessive "infantile" polycystic disease (IPCD), hereditary renal dysplasia, and hereditary tubulointerstitial nephritis. (Two other such diseases are autosomal dominant polycystic and nonsyndromatic hepatic cystic disease.)

Congenital Hepatic Fibrosis

The term "congenital hepatic fibrosis" (CHF) was adopted by Kerr, Harrison, Sherlock, and Milnes Walker (2) from Parker's paper (3) "Fibrosis of the Liver as a Congenital Anomaly." Parker had described both isolated and syndromatic hepatic fibrosis, the latter in IPCD and Meckel syndrome, and in all but one of the cases the abnormality included an excess of bile ducts. Kerr and colleagues used the term to describe a group of 12 young children and 1 teen-ager who had portal hypertension associated with hepatic fibrosis and an obvious excess of bile ducts. Many of the patients were also shown by excretory urography to have renal medullary ductal ectasia (4), an abnormality described by Reilly and Neuhauser (5) in children with cystic disease of the kidneys and liver. Medullary ductal ectasia is characteristic of IPCD (6), despite its resemblance to medullary sponge kidney. The same term, congenital hepatic fibrosis, was used by McCarthy et al (7) to describe hepatic fibrosis and bile duct hyperplasia in 10 children and adults, some of whom had portal hypertension. The term has acquired, therefore, both morphologic and clinical meanings (8). Kerr and his colleagues (2, 4) have used it to mean a syndrome of portal hypertension in patients with noncirrhotic, presumably congenital, hepatic fibrosis; Bernstein (9) has used it as descriptive of the hepatic abnormality in IPCD; and Landing and colleagues (10, 11) have used it in a more restricted morphologic fashion for a possibly separate group within the CHF-IPCD complex.

The hepatic abnormality in CHF comprises portal enlargement, portal fibrosis, an absence of central bile ducts within the portal areas, hypoplasia of portal veins, and an apparent proliferation of bile ducts (3, 6, 12, 13) (Fig. 1). The last, bile duct "proliferation," is a form of biliary dysgenesis, as it appears to be an arrested development of bile ducts rather than a proliferative reaction to biliary obstruction (13). Stereologic considerations (14) enable one to predict that the universal presence in random sections of numerous elongated bile ducts reflects saclike rather than ductlike biliary structures (12, 15–17). That anatomic arrangement had already been shown by reconstruction of the cysts (10, 18, 19), and Jørgensen (13) concluded that the bile ducts had not differentiated beyond the stage of bilaminar plates. Landing and colleagues (10) referred to the same arrangement as a perforated cylinder; that is, a three-dimensional representation of biliary dysgenesis might in simplified form be a sleeve of flattened sacs surrounding the portal tract as it ramifies through the liver. Such an arrangement would account for the occurrence in every plane of section of elongated ducts.

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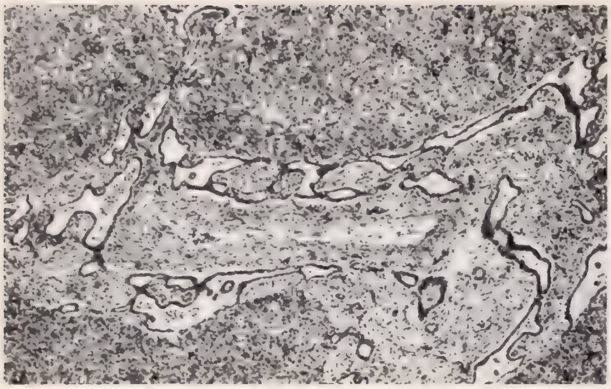


Fig. 1. Congenital hepatic fibrosis. Prototypic abnormality of congenital hepatic fibrosis in newborn infant with typical infantile polycystic disease, autosomal recessive type. Portal area is enlarged, appearing to contain large number of elongated bile ducts almost encircling portal area. Biliary sacs lie close to limiting plates of hepatic lobules, where they merge with bile ductules or canals of Hering. An interlobular bile duct cannot be identified. Portal vein (center) appears slightly compressed; hepatic artery (lower right) appears normal (H&E stain, original magnification 75×).

Biliary dysgenesis in CHF may be accompanied by gross ductal dilatation and sometimes by large intrahepatic cysts (13, 17). Nonobstructive saccular and fusiform dilatation of the intrahepatic ducts constitutes Caroli disease (20, 21). The diagnosis is usually established by cholangiography, which differentiates it from other cystic abnormalities. Although Caroli disease may occur as an isolated abnormality, without other malformations, it occurs most often in the context of renal cystic disease with medullary ductal ectasia (22).

Autosomal Recessive (Infantile) Polycystic Disease. Polycystic disease of the autosomal recessive type characteristically manifests as enlarged, spongy kidneys and severe biliary dysgenesis in newborns (Fig. 2). The renal cysts reside almost entirely within collecting ducts. The medullary pyramids contain rounded cysts that appear to be under some tension, and dilated ducts extend from the outer medulla through the thickness of cortex. Medullary ductal ectasia is the characteristic radiographic abnormality in patients of all ages. Thin, saccular dilatations reside within the medullary rays of the cortex, and microscopic examination shows the convoluted tubules and glomeruli to be relatively normal. Older children have less severe ductal dilatation in that cysts are less diffuse, sometimes only focal, although their localization is the same (Fig. 3).

I have attributed the morphologic variation to the severity of expression of the genetic disorder (9), whereas Landing and colleagues have used the morphologic variation to define two genetically different disorders, IPCD and CHF (11). The renal cysts in patients classified as CHF by Landing et al (11) are more centrally located in the kidney and are associated with more slowly progressive disease than are the cysts in the patients they classified as IPCD (23).

The urographic appearance of ductal ectasia may be indistinguishable from that of medullary sponge kidney, although sonography demonstrates the renal enlargement and typical hepatic echogenicity of IPCD-CHF (24). The two conditions have different clinical, genetic, and morphologic characteristics. Progression of IPCD in

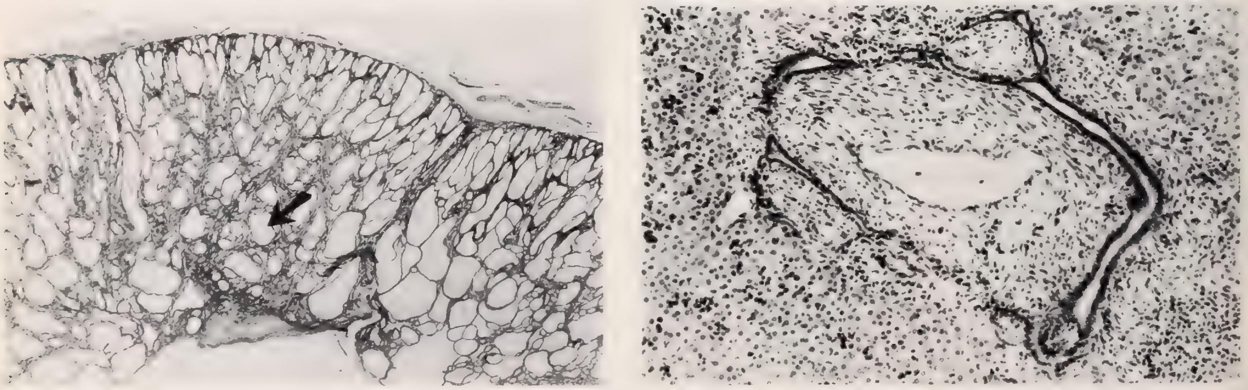


Fig. 2. Infantile polycystic disease in newborn. **Left** Kidney appears spongy because of dilated collecting ducts, elongated in cortex and somewhat rounded in medulla (arrow). Usual relationships of cortical medullary rays and cortical labyrinths have been obscured in this specimen, and corticomedullary junctions are indistinct (H&E stain, original magnification 6×). **Right** Liver (same patient) contains minimally enlarged portal areas ringed by flattened biliary sacs that in two dimensions appear as interconnecting elongated bile ducts (H&E stain, original magnification 120X).

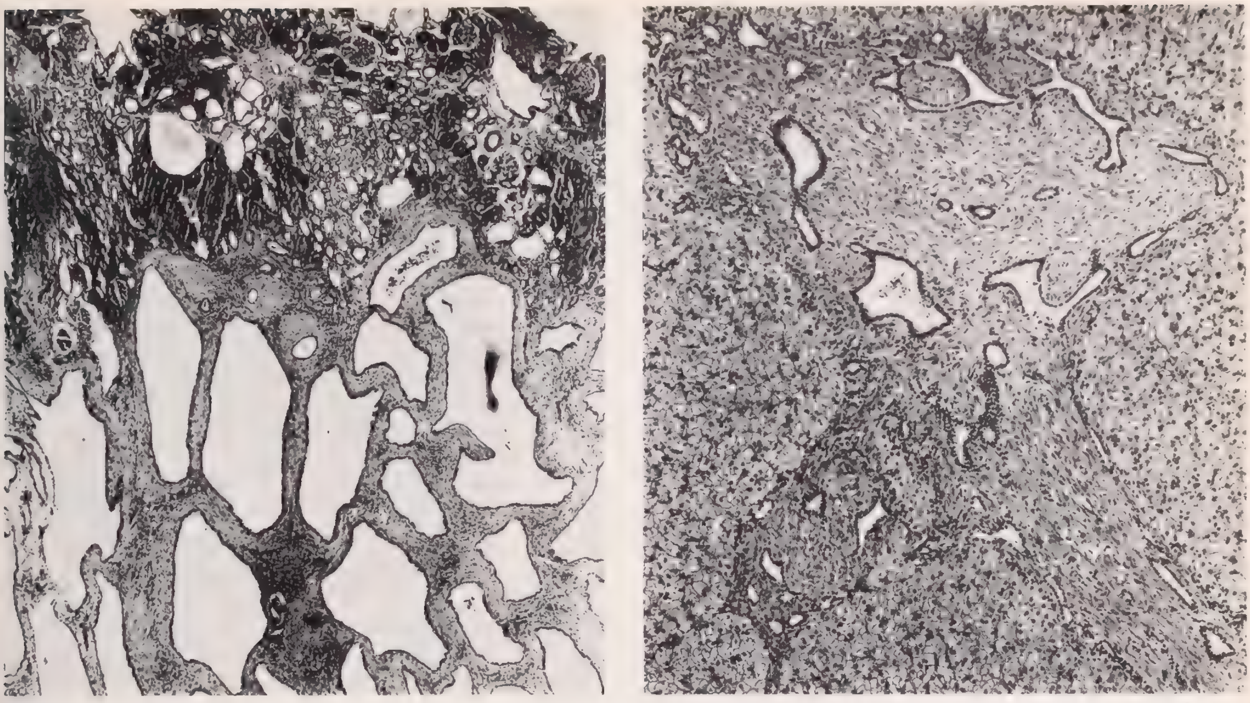


Fig. 3. Infantile polycystic disease in older child. **Left** Kidneys are less diffuse and cystic than in newborn, with small cortical cysts along the medullary rays and affecting nephrons within cortical labyrinths. Collecting ducts within medulla are severely ectatic, with fibrosis of surrounding medullary tissue (H&E stain, original magnification 12.5×). **Right** Hepatic abnormality similar to that in newborn; increased proportion of fibrous tissue in relation to biliary structures, which appear as irregularly interrupted, flattened sacs, some ringing portal areas (H&E stain, orignal magnification 25×).

patients with initially mild expression seems to result from progressive tubular atrophy rather than from progressive cyst formation, although Dupond et al (25) have demonstrated the occasional late occurrence of diffuse cystic disease.

The hepatic abnormality in IPCD, the prototype of CHF and biliary dysgenesis, is characterized by portal enlargement with apparent bile duct proliferation. That increase in biliary structures reflects, as noted above, an anatomic arrangement of flattened sacs or cisterns. The biliary spaces in IPCD sometimes form almost continuous, irregularly interrupted rings around the peripheries of the portal areas, but the arrangement is often considerably more irregular, with branches extending into or tributaries coming out of the hepatic lobules. Furthermore, dilatation of biliary sacs is often present at birth, with no evidence of obstruction.

Patients with CHF and biliary dysgenesis are seldom jaundiced, even when morphologic studies show localized filling of the biliary passages with inspissated bile, and the development of jaundice usually indicates a complication such as ascending infection or superimposed hepatocellular injury. Patients with CHF, especially those with Caroli disease, carry a high risk of biliary infection (26), either cholangiolitis or suppurative cholangitis. The two complications, localized plugging and acquired infection, may contribute to the evolution of the abnormality. Although increasing fibrosis appears to be an intrinsic part of the disease (27, 28), severe portal fibrosis and perilobular fibrosis with a pattern barely distinguishable from micronodular cirrhosis probably result also from localized biliary obstruction and especially from infection.

Hereditary Renal Dysplasia. A hepatic abnormality almost identical to that in CHF-IPCD occurs in several syndromes of heritable renal

TABLE 1

Hereditary Renal Dysplasia with Congenital Hepatic Fibrosis and Biliary Dysgenesis

- Meckel syndrome and congeners
- Chondrodysplasia syndromes
  - Majewski and Saldino-Noonan short rib-polydactyly syndromes
  - Jeune asphyxiating thoracic dysplasia
  - Ellis-van Creveld chondroectodermal dysplasia
  - Elejalde acrocephalopolydactylous dysplasia
- Trisomy C (trisomy 9) syndrome
- Trisomy D (trisomy 13) syndrome
- Zellweger cerebrohepatorenal syndrome
- Ivemark renal-hepatic-pancreatic dysplasia
- Glutaric aciduria, type II

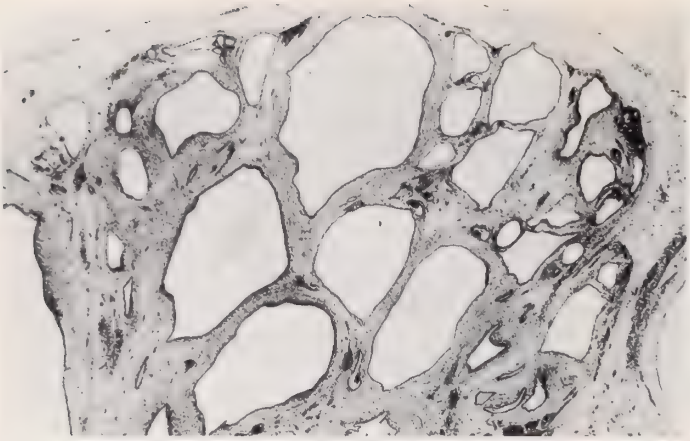


Fig. 4. Meckel syndrome. **Left** Renal dysplasia in stillborn infant with occipital encephalocele, polydactyly, and cystic liver. Rudimentary renal lobe contains dilated primitive ducts, branches of ureteric bud. Minimal metanephric differentiation has resulted in formation of a few primitive tubules and glomeruli, with consequent lack of corticomedullary differentiation (H&E stain, original magnification 16×).

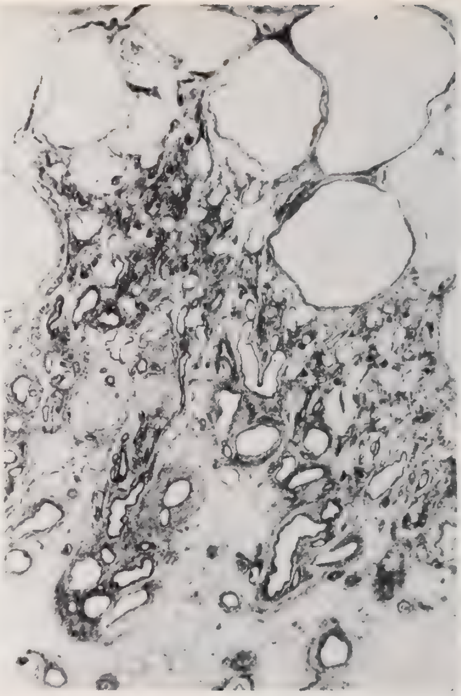


Fig. 5. **Right** Meckel syndrome. Another example of renal cystic dysplasia: greater nephronic differentiation within inner cortex. Cysts present in outer cortex; rudimentary medulla consists only of small clusters of primitive ducts (H&E stain, original magnification 16×).

dysplasia. Almost every heritable syndrome of renal dysplasia also includes biliary dysgenesis (Table 1). The renal abnormalities are all very similar—diffuse cystic dysplasia with abnormal ductal differentiation, deficient nephronic differentiation, and poor lobar organization (29) (Fig. 4). Lobar disorganization results from the abnormal ductal and nephronic differentiation and is characterized by small medullary pyramids that lack recurrent loops and vasa recta and are barely demarcated from surrounding pelvic tissue (Fig. 5). The cortices lack the customary arrangement of cortical labyrinth surrounding the medullary rays; that is, the cortices lack the usual arrangement in which clusters of glomeruli and convoluted tubules alternate with bundles of ducts and blood vessels, and there may be nests of metaplastic cartilage. The malformed kidneys are seldom able to support renal function and to maintain life, and most cases are recognized postmortem in newborns and very young infants.

The best known of the syndromes of renal cystic dysplasia is Meckel syndrome, which typically includes biliary dysgenesis (Fig. 6), occipital encephalocele, cleft lip, polydactyly, and abnormal genitalia. Phenotypic expression in Meckel syndrome is extremely variable, however, and the renal abnormality is the most consistent malformation in the syndrome (30–32). Although Meckel syndrome is not common, the other syndromes of heritable renal dysplasia are even less common, and their identification depends on the pattern of associated malformations. Some, like Zellweger and Jeune syndromes, only occasionally include renal cystic dysplasia (29) (Fig. 7). The renal abnormality in Zellweger cerebrohepatorenal syndrome more often takes the form of scattered, inconsequential cortical microcysts (33, 34), and the principal renal abnormality in Jeune asphyxiating thoracic dystrophy is a chronic, progressive tubulointerstitial nephritis (35–38).

Whether the cases of Jeune and Zellweger syndromes with renal dysplasia constitute, because of the different phenotype, evidence of genetic heterogeneity is not clear; Leclerc et al (39) thought their case of "hepato-pancreato-epididymo-renal polycystosis with thoracic polydystrophy" to be different from Jeune's despite the similarities. Both Zellweger and Jeune syndromes include biliary dysgenesis (Fig. 8). Other forms of chondrodysplasia regularly associated with renal dysplasia and biliary dysgenesis are Majewski and Saldino-Noonan short rib–polydactyly syndromes (40) and Elejalde acrocephalopolydactylous dysplasia (41). Ellis-van Creveld chondroectodermal dysplasia syndrome has been reported to include renal dysplasia and biliary dysgenesis (42). Trisomy C (trisomy 9) is clearly associated with both abnormalities (43, 44), and

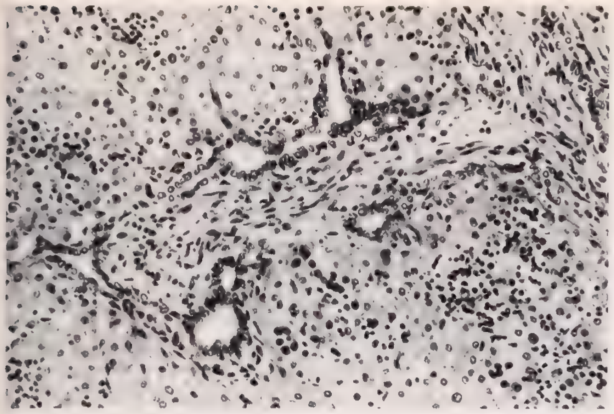


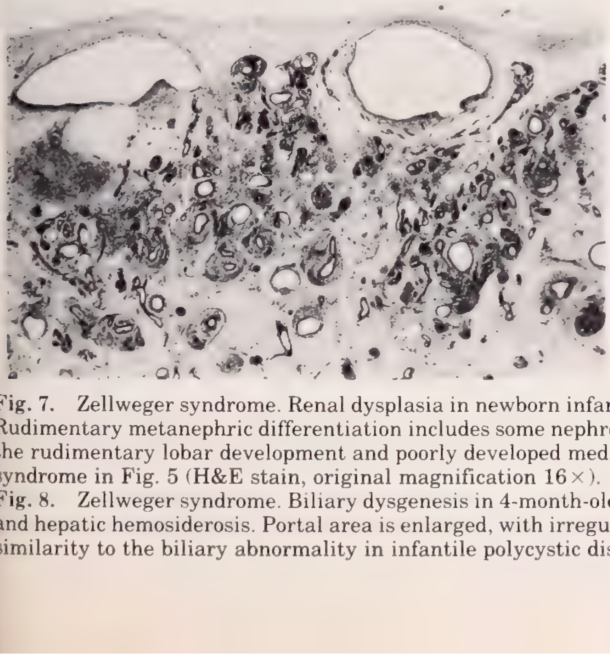
Fig. 6. Meckel syndrome. Biliary dysgenesis in one of two affected sibs. Biliary passages are almost continuous around periphery of portal area, receiving tributaries from within hepatic lobules (H&E stain, original magnification 160×).

trisomy D (trisomy 13) is so occasionally (45). Renal dysplasia and biliary dysgenesis are among the malformations occurring in glutaric aciduria type II (46). There is, however, considerable overlap among the syndromes, with frequent involvement of the pancreas, brain, eye, genitalia, extremities, bones, and face (Table 2), and syndrome recognition, beyond the scope of this presentation, involves careful consideration of clinical, genetic, radiographic, and morphologic findings.

Biliary dysgenesis, in association with heritable renal dysplasia, differs little from biliary dysgenesis in IPCD. The differences appear to be matters of degree rather than of character, with less severe bile duct "proliferation" and less severe portal fibrosis. The biliary passages more often have an irregular pattern, but the essential saclike structure is the same (47). Children do not live long enough to develop portal hypertension, but they do get biliary ectasia with a pattern indistinguishable from Caroli disease and they do get large intrahepatic cysts. We have not encountered ascending infection, but the possibility is not to be ruled out.

Hereditary Tubulointerstitial Nephritis. Biliary dysgenesis has been identified in three varieties of hereditary tubulointerstitial nephritis. The most common perhaps is juvenile nephronophthisis (Fig. 9), which was recognized by Boichis et al (48) to be associated with a hepatic abnormality very similar to congenital hepatic fibrosis (Fig. 10). These patients, surviving into the second decade of life, probably have the potential for developing portal hypertension; gross cysts and ascending infection, although not yet described, are not to be ruled out. The renal abnormality consists principally of tubular atrophy with interstitial fibrosis, accompanied by mild interstitial chronic inflammation and secondary glomerular sclerosis. Medullary cysts, a common feature of juvenile nephronophthisis, are often lacking in patients with hepatic fibrosis; glomerular cysts, uncommon in other types of juvenile nephronophthisis, are frequently present (48).

Other forms of hereditary tubulointerstitial nephritis with hepatic involvement include Bardet-Biedl (49, 50) and Jeune syndromes (37). The renal abnormality in Jeune syndrome may, as noted, occasionally take the form of cystic dysplasia or diffuse cystic disease, but the most



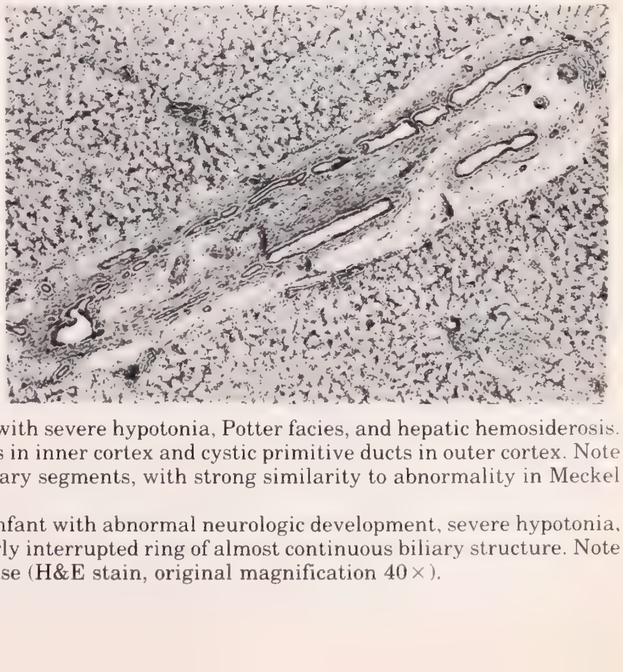
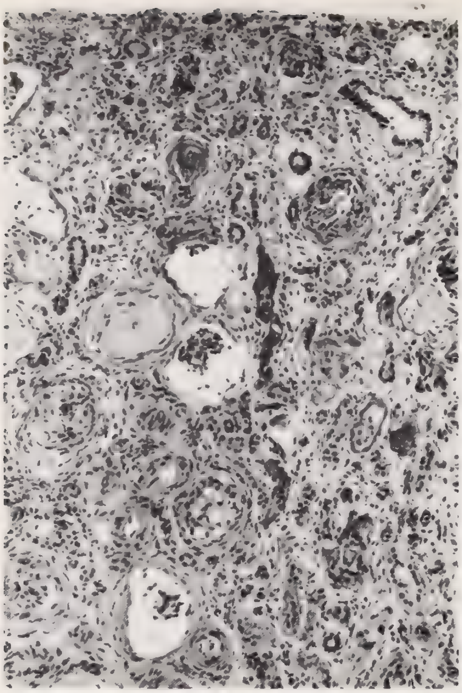


Fig. 7. Zellweger syndrome. Renal dysplasia in newborn infant with severe hypotonia, Potter facies, and hepatic hemosiderosis. Rudimentary metanephric differentiation includes some nephrons in inner cortex and cystic primitive ducts in outer cortex. Note the rudimentary lobar development and poorly developed medullary segments, with strong similarity to abnormality in Meckel syndrome in Fig. 5 (H&E stain, original magnification 16×).

Fig. 8. Zellweger syndrome. Biliary dysgenesis in 4-month-old infant with abnormal neurologic development, severe hypotonia, and hepatic hemosiderosis. Portal area is enlarged, with irregularly interrupted ring of almost continuous biliary structure. Note similarity to the biliary abnormality in infantile polycystic disease (H&E stain, original magnification 40×).



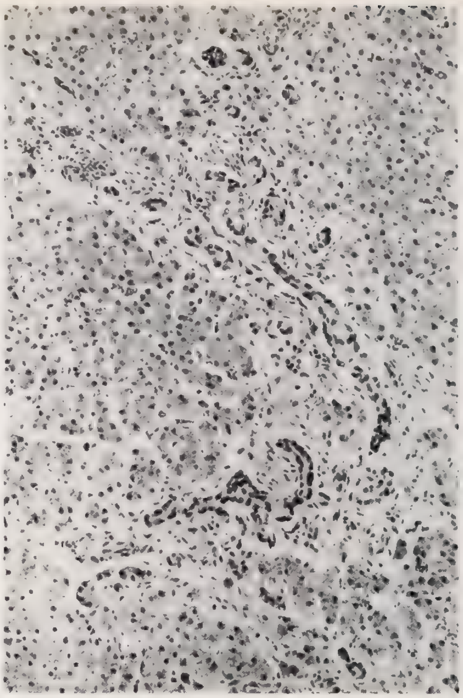


Fig. 9. Juvenile nephronophthisis. Tubulointerstitial nephritis in 2½-year-old boy who had renal failure at a few months of age. Severe tubular atrophy with secondary glomerular obsolescence (H&E stain, original magnification 100×).

Fig. 10. Biliary dysgenesis and hepatic fibrosis in same patient. Portal area is moderately enlarged and contains excessive, elongated biliary profiles (H&E stain, original magnification 100×).

common renal abnormality is a tubulointerstitial nephritis with features indistinguishable from those of juvenile nephronophthisis (38). The renal abnormality in Bardet-Biedl syndrome is also variable, most often a tubulointerstitial nephritis with medullary cysts, sometimes an apparently primary glomerular disease (51).

Other abnormalities in these three syndromes include obesity, mental retardation, retinal dysplasia, polydactyly, skeletal dysplasia, and central nervous system abnormalities in combinations with considerable overlap, resulting in a somewhat difficult differential diagnosis (Table 3).

Summary

Malformations of the liver and kidney often coexist. Several heritable syndromes, including polycystic disease, renal dysplasia, and tubulointerstitial nephritis, also affect the liver to result in biliary dysgenesis, an abnormality of bile duct

TABLE 2

Associated Malformation in Principal Syndromes of Hereditary Renal Dysplasia

Syndrome	Bil	Panc	CNS	Ocu	Gen	Poly	Chon	CP
Meckel	+	+	+	+	+	+	(+)	+
Chondrodysplasia								
Short rib-polydactyly	+	+	+		+	+	+	+
Jeune (asphyxiating thoracic dysplasia)	+	+		+	+	+	+	
Trisomy C	+	+	+	+			(+)	+
Ivemark (renal-hepatic-pancreatic dysplasia)	+	+		+	+			
Zellweger (cerebrohepatorenal)	+		+	+	(+)		+	
Glutaric aciduria, type II	+	+	+					

Bil, biliary dysgenesis; Panc, pancreatic dysplasia; CNS, central nervous system maldevelopment; Ocu, ocular abnormalities; Gen, genital abnormalities; Poly, polydactyly; Chon, chondrodysplasia; CP, cleft palate.

+ = Present; (+) = questionably present.

TABLE 3
Associated Abnormalities in Principal Syndromes of Hereditary Tubulointerstitial Nephritis

Syndrome	Obe	Ment	Ret	Poly	Skel	CNS	Hep
Familial juvenile nephronophthisis	+	+	+	+	+	+	+
Bardet-Biedl	+	+	+	+		+	+
Jeune (asphyxiating thoracic dysplasia)		(+)	+	+	+		+

Obe, obesity; Ment, mental retardation; Ret, retinal dysplasia; Poly, polydactyly; Skel, skeletal dysplasia; CNS, central nervous system abnormalities; Hep, hepatic fibrosis.
+ = Present; (+) = questionably present.

differentiation, and congenital hepatic fibrosis. The hepatic portal areas are enlarged and fibrotic, appearing to contain an excess of elongated bile ducts. The biliary structures can be assumed on stereologic grounds, confirmed by morphologic reconstructions, to be in the form of flattened sacs rather than ducts. Congenital hepatic fibrosis in all its forms is complicated by nonobstructive biliary ectasia (Caroli disease). Portal hypertension and ascending infection are well-known complications of autosomal recessive (infantile) polycystic disease with congenital hepatic fibrosis, and prolonged survival of children with the other entities mentioned may unmask similar complications.

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The Classification of Vasculitis and a Reappraisal of Allergic Granulomatosis and Angiitis (Churg-Strauss Syndrome)

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Jacob Churg and Lotte Strauss were both past recipients of the Jacobi Medallion, awarded in recognition of their contributions to medicine by the Mount Sinai School of Medicine, New York City. Dr. Strauss was a pioneer in pediatric and perinatal pathology, and she also trained scores of pathologists in that specialty. Although perhaps best known for his work related to renal disease, Dr. Churg is an acknowledged authority in virtually every branch of anatomic pathology. The 1964 publication "Asbestos Exposure and Neoplasia" (1), of which Dr. Churg was a coauthor, has often been singled out as a landmark article on environmental health and occupational disease (2). However, it was the clinicopathologic entity of "allergic granulomatosis and angiitis" described by Churg and Strauss in 1951 (3) that earned the eponym of Churg-Strauss syndrome. Churg-Strauss syndrome, albeit generally perceived to be uncommon, is a legitimate member of the expanding clinical spectrum of systemic vasculitis.

Morphologic Classification of Vasculitis: A Historical Perspective

Vasculitides and aneurysms are diseases of antiquity. Aneurysm was the only form of arterial disease known to the ancient physicians. Galen classified aneurysm, together with the varix, as a kind of *tumor contrary to nature*—an iatrogenic lesion; the lesion would develop if the physician, in bloodletting, had lacerated the artery instead of the vein. Eponyms are common among vasculitides, but not all are correctly assigned.

Syphilitic aortitis and aneurysm was probably first described by Anatoine Saporta in 1554 when syphilis swept over Europe in the 16th century (4). Almost three hundred years later, Schönlein (5), in 1837, described the vascular purpura that was later to bear his name. Savory (6), in 1856, was probably the first to describe the disease that unjustly bears someone else's name: Takayasu arteritis (7). Felix von Winiwarter (8), in 1879, described thromboangiitis obliterans, which is better known outside Europe as Buerger's disease (9). The description by Jonathan Hutchinson in 1890 (10) of "thrombotic arteritis of the aged" affecting an 80-year-old man who had painful swellings in his temples can readily be identified as temporal arteritis. The histopathology of temporal arteritis and the clinical syndrome related to it were first recognized by Horton and associates (11–13).

Necrotizing Vasculitides. As the prototype of systemic necrotizing vasculitis, "periarteritis nodosa" was first described in 1866 by Adolf Kussmaul, the clinician, and Rudolf Maier, the pathologist (14). Ferrari (15) introduced the alternative name *polyarteritis,* pointing out that the arterial inflammation was more often transmural than merely in the outer wall. Involvement of pulmonary arteries in polyarteritis was subsequently described by Mönckeberg (16), in 1905, and by Ophüls (17), in 1923, as were necrotizing lesions of veins.

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TABLE 1

Typological Classification of Vasculitis by Predominant Size of Blood Vessel Affected

Large and medium-sized
- Syphilitic arteritis
- Takayasu's arteritis
- Granulomatous giant cell arteritis
  - Cranial giant cell (temporal) arteritis
  - Extracranial giant cell arteritis
  - Disseminated visceral giant cell angiitis
  - Granulomatous angiitis of the central nervous system

Medium-sized and small
- Thromboangiitis obliterans (Buerger's disease)
- Polyarteritis nodosa
  - Adult classic and microscopic forms
  - Cutaneous polyarteritis/isolated polyarteritis
  - Infantile polyarteritis (Kawasaki's disease)
- Pathergic-allergic granulomatosis and angiitis
  - Wegener's granulomatosis
  - Churg-Strauss syndrome
  - Necrotizing sarcoid granulomatosis
  - (Polymorphic reticulosis; lymphomatoid granulomatosis)
- Vasculitis of collagen-vascular disease
  - Rheumatic fever
  - Rheumatoid arthritis
  - Seronegative arthritis
  - Dermatopolymyositis
  - Scleroderma
  - Systemic lupus erythematosus
  - Relapsing polychondritis
  - Sjögren syndrome
  - Behçet syndrome
  - Cogan syndrome

Small
- Hypersensitivity vasculitis (of Zeek)
- Leukocytoclastic vasculitis
- Schönlein-Henoch purpura
- Mixed cryoglobulinemia
- Hypocomplementemia
- Malignancies

TABLE 2

*Cutaneous Extravascular Necrotizing Granuloma (Churg-Strauss Granuloma) and Systemic Diseases**

Vasculitides	16	
Churg-Strauss syndrome		7
Wegener's granulomatosis		3
Polyarteritis nodosa		1
Takayasu arteritis		1
Cutaneous vasculitis		1
Vasculitis (unclassified)		3
Connective tissue disease	6	
Rheumatoid arthritis		4
Systemic lupus erythematosus		2
Lymphomyeloproliferative disease	3	
Acute myelomonocytic leukemia		1
Multiple myeloma		1
Lymphocytic lymphoma		1
Miscellaneous immunoreactive disorder	3	
Bacterial endocarditis		1
Chronic active hepatitis		1
Chronic ulcerative colitis		1

* Modified from Finan and Winkelmann (55).

In 1926, von Glahn and Pappenheimer (18) observed vascular lesions in rheumatic disease but thought they were different from periarteritis nodosa. Rössle (19), in 1933, attempted to include rheumatic carditis and periarteritis in a circle of rheumatic collagen diseases. This circle consisted of diseases which present a combination of typical rheumatic lesions and necrotizing vascular lesions of the periateritis nodosa type, in varying proportions and combinations. One of Rössle's patients (case IV) actually presented a granulomatous and necrotizing vascular lesion with marked involvement of the respiratory tract. This might have been an early example of the as yet unnamed entity of Churg-Strauss syndrome or Wegener's granulomatosis. Only 2 years earlier, Klinger (20), in 1931, had described a patient with destructive sinusitis, nephritis, and disseminated vasculitis. Klinger considered this to be a borderline case of periarteritis nodosa, but we now accept it as the first example of Wegener's granulomatosis. Wegener's reports (21, 22) of "rhinogenic granuloma" were published in 1936 and 1939.

Thus, in the ensuing decades after the description by Kussmaul and Maier, in 1866 (14), of periarteritis nodosa, the etiology of this vascular disease remained unknown, and the morphologic and clinical picture of true periarteritis has lost its clarity. Periarteritis (or polyarteritis) nodosa soon became the designation for almost any necrotizing vasculitis in which the etiology could not be established.

The suggestion by Gruber in 1925 (23) that hypersensitivity might be involved in the production of vascular lesions in periarteritis later received support from experimental animal studies of sensitization to foreign proteins (24) and from the clinical observation of patients allergic to sulfonamides (25). However, hypersensitivity or allergic vasculitis often affects the blood vessels that are seldom involved in periarteritis, such as the small arteries and veins, and arterioles and venules. Whether any morphologic similarity of hypersensitivity angiitis to the vascular lesion of periarteritis nodosa constitutes proof of a common etiology is questionable.

Competing Classifications. The stage was then set for a classification of necrotizing vascular lesions to lessen the confusion in the nosology of vasculitides. Zeek, in 1952 (26, 27), proposed the generic term "necrotizing angiitis" to designate the vascular lesions, both arterial and venous, the fully developed stage of which consisted of fibrinoid necrosis and inflammatory reaction involving all three coats of vessel walls. Five types of necrotizing angiitis were differentiated and designated as follows: (1) hypersensitivity angiitis, (2) allergic granulomatous angiitis, (3) rheumatic arteritis, (4) periarteritis nodosa, and (5) temporal arteritis. Zeek was apparently either unacquainted with Wegener's granulomatosis or had grouped it together with allergic granulomatous angiitis in this classification.

Expanding on the scheme devised by Zeek (26, 27), there have been numerous other attempts to classify vasculitis according to the size and type of vessels affected, the presence or absence of lung involvement, the nature of cutaneous or renal lesions, and the presumptive etiologic factors and pathogenesis (28–38), none of which can be entirely satisfactory so long as the etiology and pathogenesis of most vasculitides are either unknown or incompletely understood. The prevailing theory is that most vasculitic syndromes are caused by deposition of immune complexes in vessel walls (29–32). The probable antigen has been identified in very few instances, notably in some patients with hepatitis B infection (28). Moreover, the immune-complex-mediated tissue injury does not produce a single or stereotypic clinical syndrome, but it tends to apply to the whole spectrum of vasculitides and

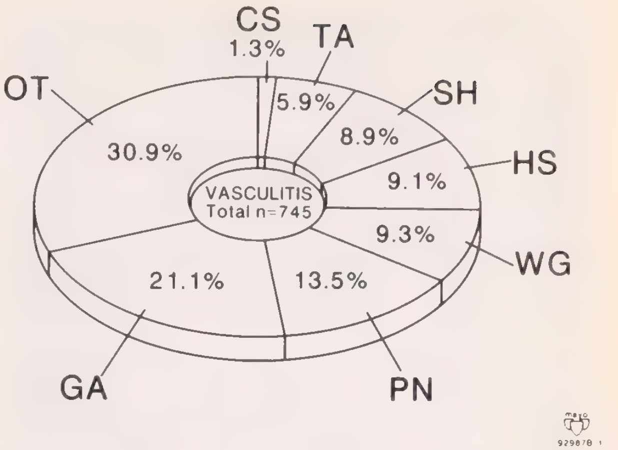

Fig. 1. Distribution of eight major types of vasculitis among random collection of 745 cases. CS, Churg-Strauss syndrome (10 cases); TA, Takayasu arteritis (44 cases); SH, Schönlein-Henoch purpura (66 cases); HS, hypersensitivity angiitis (68 cases); WG, Wegener's granulomatosis (69 cases); PN, polyarteritis nodosa (101 cases); GA, giant cell temporal arteritis (157 cases); OT, others, type unspecified (230 cases).

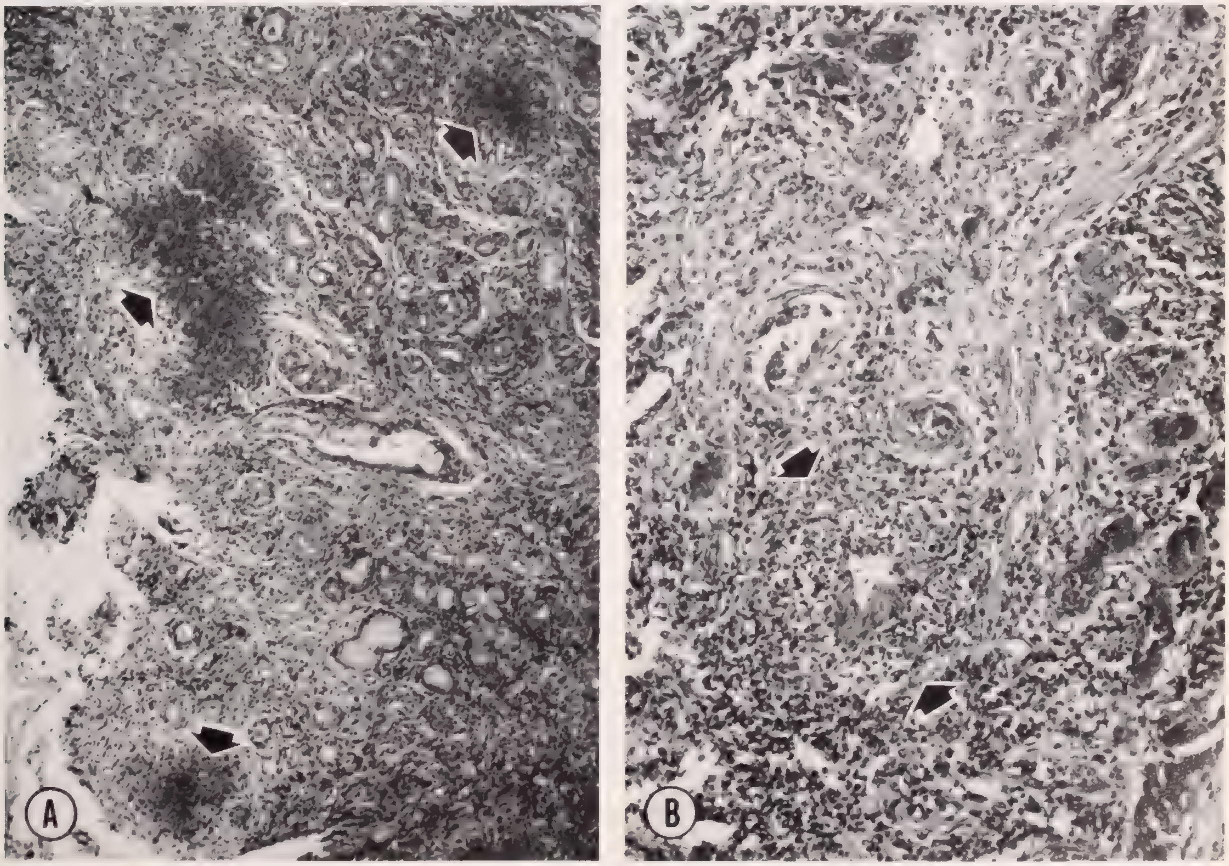

Fig. 2. Pulmonary lesions in Churg-Strauss syndrome. (A) Eosinophilic granulomas (arrows) in a bronchial biopsy; (B) necrotizing vasculitis (arrows) with predominance of eosinophils in the cell infiltrate. (Hematoxylin and eosin, A ×100; B ×160.)

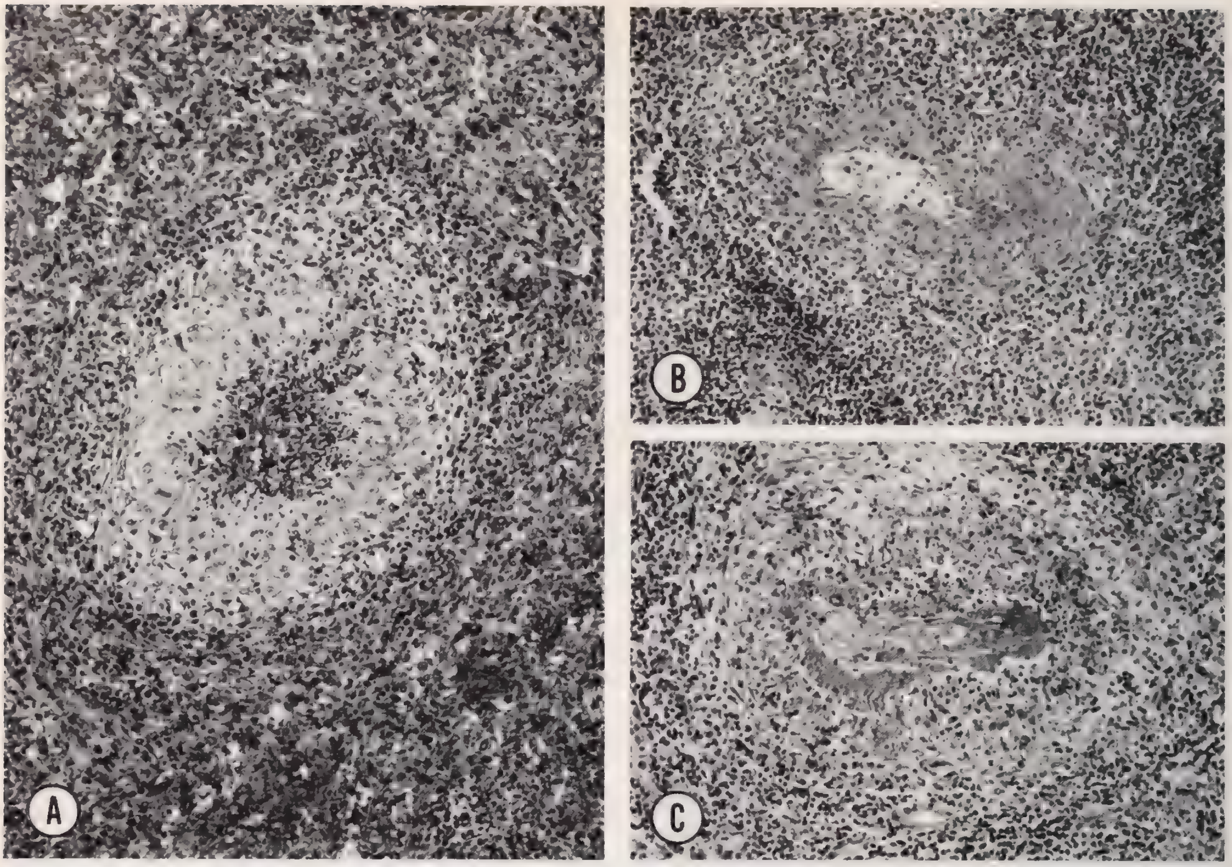

Fig. 3. Vascular and extravascular lesions of spleen in Churg-Strauss syndrome. (A) Typical eosinophilic granuloma with necrotic center surrounded by radially aligned epithelioid cells and occasional giant cells; (B and C) necrotizing vasculitis with fibrinoid necrosis and eosinophil-rich cell infiltrate. (Hematoxylin and eosin, ×160.)

to overlap with injuries caused by other immune mechanisms.

With the proliferation of competing schemes of classification, it becomes inevitable that the same name has been used to refer to different vasculitic syndromes and different names are adopted to describe the same condition. Zeek (26, 27) had originally used the term "hypersensitivity angiitis" to refer to patients with disseminated necrotizing vasculitis of arterioles and venules, both systemic and pulmonary, often with renal and cutaneous involvements. The same term has now been used by others (27, 39) to refer to conditions in which cutaneous vasculitis dominates the clinical picture and visceral involvement is absent or occurs only rarely. Just as confusing is the adoption of the names "allergic vasculitis" (40, 41) and "leukocytoclastic vasculitis" (42, 43) as synonyms for Zeek's hypersensitivity angiitis.

In the past, an exact characterization of different types of vasculitic syndromes might have been principally a matter of academic interest since few effective therapeutic modalities existed. We now know that for many severe forms of vasculitis, such as Wegener's granulomatosis and systemic necrotizing vasculitis, the outlook may be vastly improved and long-term remissions made possible by treatment with corticosteroids and/or cyclophosphamide (44–47). Early and correct diagnosis of vasculitis becomes vital not only for prompt and aggressive immunosuppressive treatment of certain malignant forms of vasculitis to prevent irreversible tissue damage, but also for withholding cytotoxic agents in situations where their use is unwarranted.

Since few, if any, of the vasculitic syndromes have pathognomonic clinical and laboratory features, histologic evaluation of the appropriate tissue remains the definitive means of establishing the diagnosis of vasculitis. Table 1 is an expanded and modified version of Zeek's histopathologic classification of systemic vasculitis, based on the predominant type and size of vessels affected. Incomplete and imperfect though this scheme may be, it offers the clinician guidelines for when to suspect and what to expect in the biopsy diagnosis of vasculitis.

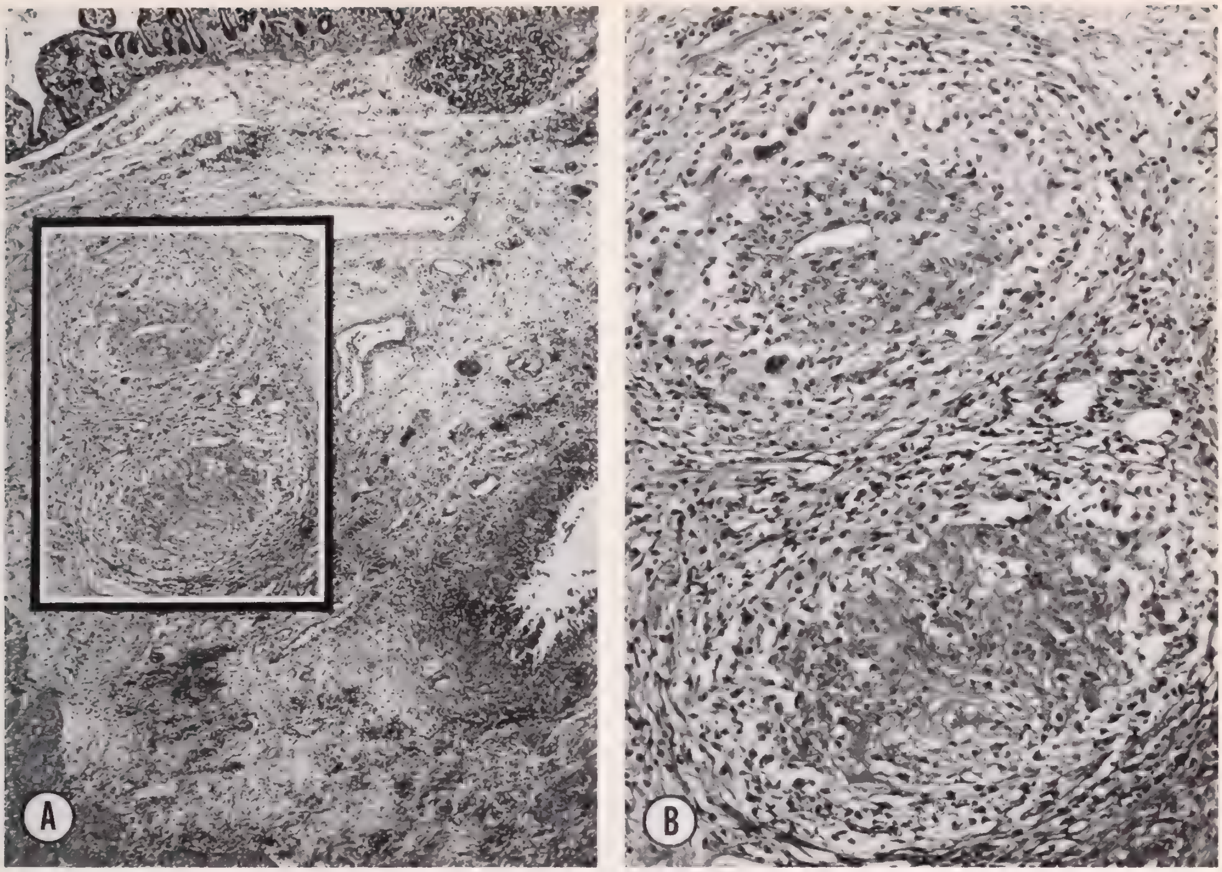

Fig. 4. Small-bowel biopsy in Churg-Strauss syndrome. (A) Granulomatous and necrotizing vasculitis in submucosal artery and vein. (B) Close-up view of boxed area in *A*. (Hematoxylin and eosin, A ×64; B ×160.)

Allergic Granulomatosis and Angiitis (Churg-Strauss Syndrome)

Allergic granulomatosis and angiitis, or Churg-Strauss syndrome, is a systemic disease that closely resembles polyarteritis and has three distinct clinical features: a history of asthma, peripheral eosinophilia which may exceed 80%, and pulmonary involvement in one-third to one-half of the reported cases. The entity is called allergic not because the immunopathologic mechanism of the disease is known but because it occurs in patients with allergic diathesis.

The original group of 13 cases assembled and described by Churg and Strauss (3) were characterized by well-defined histopathologic lesions: necrotizing vasculitis, extravascular granulomas, and tissue eosinophilia (3, 48, 49). The same authors (50) later widened their concept of allergic granulomatosis and angiitis to include such diverse entities as eosinophilic pneumonitis, eosinophilic nonnecrotizing angiitis, bronchocentric granuloma, allergic granuloma, and necrotizing angiitis. In the series of 30 cases reported by Chumbley and associates (51), allergic granulomatosis of the prostate and lower urinary tract was added to the systemic manifestations of Churg-Strauss syndrome. The concept of a localized, isolated, or limited form of Churg-Strauss syndrome is gaining support (52–54), and the typical cutaneous extravascular granuloma (Churg-Strauss granuloma) has been observed in a range of disparate conditions (55) (Table 2). There is also a report of the association of Churg-Strauss syndrome with temporal arteritis, primary biliary cirrhosis, and polychondritis in a single patient (56).

How Rare Is It? In addition to the original autopsy series of 13 cases described by Churg and Strauss (3) and scattered single case reports (57), only one other series of 30 cases from our institution collected over a 25-year period (51) has appeared in the literature, giving the impression that Churg-Strauss syndrome is rare. This apparent rarity of Churg-Strauss syndrome is misleading because a large number of similar or identical conditions have gone unrecognized or been recorded under different names. Adopting

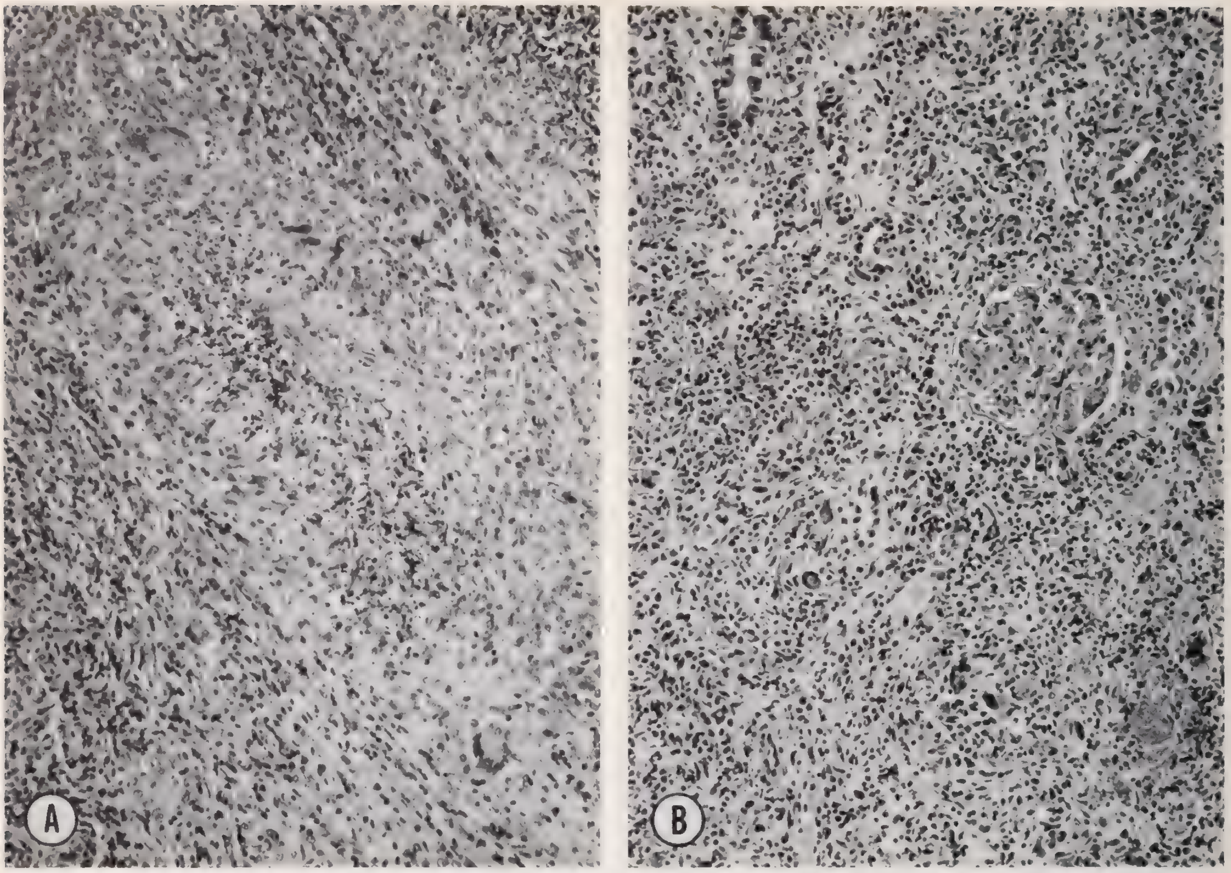

Fig. 5. Renal lesions in Churg-Strauss syndrome. (A) Granulomatous vasculitis of interlobar artery; (B) focal necrotizing glomerulitis and necrotizing granulomas. (Hematoxylin and eosin, ×160.)

the following three criteria: asthma, peak peripheral blood eosinophil counts in excess of 1.5 × $10^3/\mu l$, and systemic vasculitis involving two or more extrapulmonary organs, Lanham and associates (57) were able to identify 138 cases of Churg-Strauss syndrome in the English literature surveyed to June 1982, plus 16 cases of their own in one hospital. Churg-Strauss syndrome is most often disguised or reported as polyarteritis associated with asthma, polyarteritis with pulmonary involvement, Löffler syndrome, eosinophilic pneumonitis or eosinophilic gastroenteritis (57), and hypereosinophilic syndrome with pulmonary vascular involvement (58).

Churg and Strauss (3) freely acknowledged that their report dealt with cases described by Rackemann and Greene (59) as "periarteritis nodosa and asthma" and by Harkavy (60) as "vascular allergy." Two reports from our institution, prior to that of Chumbley and associates (51), also dealt with the subgroup of 28%–32% of patients who had periarteritis nodosa with pulmonary involvement (61, 62). Rose and Spencer (63), in their analysis of 111 autopsy cases of polyarteritis nodosa, also recognized 32 cases (29%) having pulmonary involvement with features similar to those described by Churg and Strauss (3). Although it was said in a 1984 CPC discussion (64) that no case of Churg-Strauss syndrome has been seen at the Massachusetts General Hospital, one can find at least six CPC cases, going back to 1939 (64–69), that would have fulfilled the clinical criteria of Churg-Strauss syndrome (57).

As might be expected, most of the cases reported by Churg and Strauss (3) were also indexed under the heading of periarteritis nodosa in the hospital records, but all 13 patients at one time had asthma and peripheral blood eosinophilia. Eosinophilia seldom occurs in patients with periarteritis nodosa unassociated with asthma or a history of allergy. Wilson and Alexander (70), on reviewing the literature, found 18% of patients among 300 cases of periarteritis nodosa had asthma. They also found that eosinophilia was present in 94% of periarteritis associated with asthma and in only 6% of those without asthma. Lanham and associates (57) suggest that Churg-Strauss syndrome has often gone undiagnosed not only because of the narrow

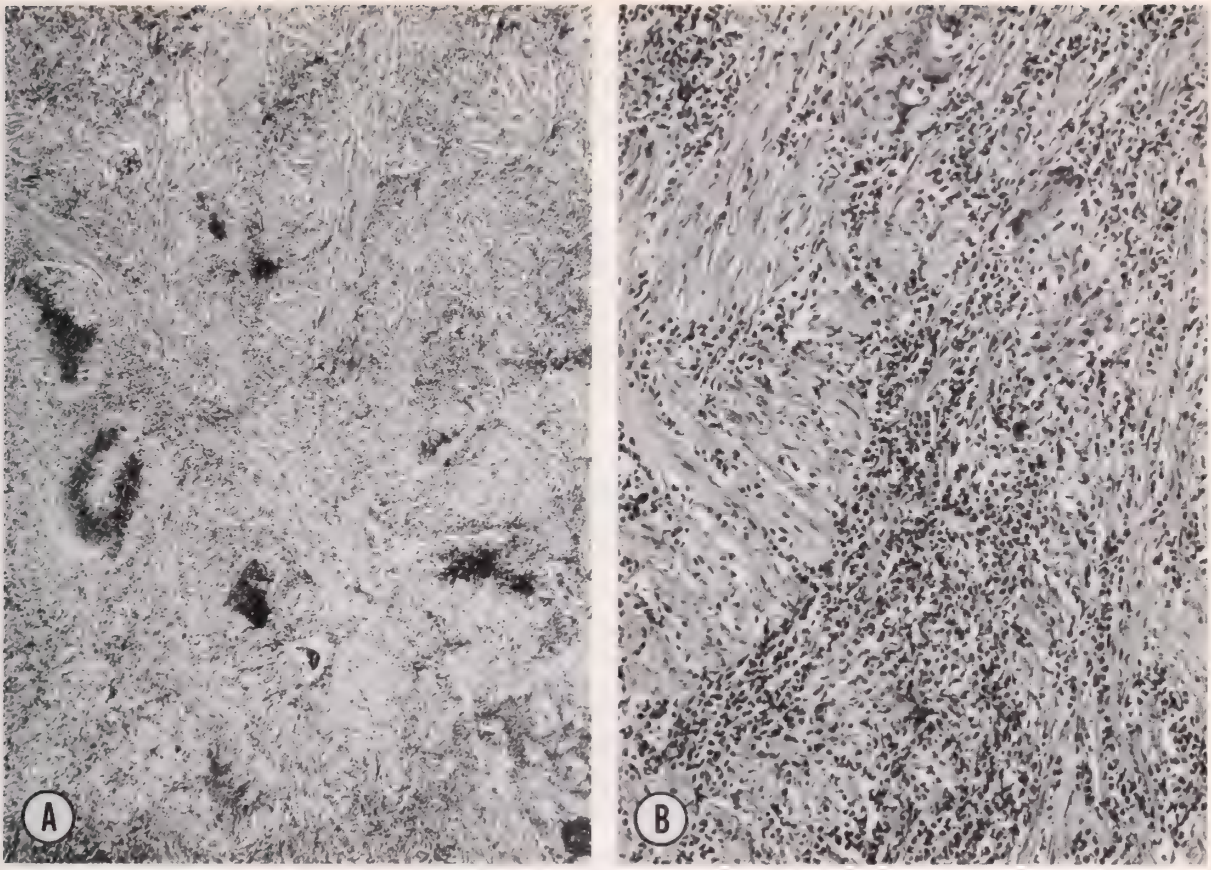

Fig. 6. (A) Eosinophilic granulomas of prostate in Churg-Strauss syndrome, morphologically indistinguishable from allergic granulomas seen in bronchial biopsy of same patient shown in Fig. 1. (B) Close-up view of granulomatous prostatitis. (Hematoxylin and eosin, A ×40; B ×160.)

focus on fulfilling the histopathologic criteria, but also because different disease entities that form components of the syndrome are recognized individually but remain unintegrated into Churg-Strauss syndrome.

Differentiating It from Wegener's Granulomatosis. The three histopathologic components of Churg-Strauss syndrome—necrotizing vasculitis, extravascular granulomas, and tissue eosinophilia—do not necessarily coexist temporally or spatially, nor are these features pathognomonic of allergic granulomatosis and angiitis. The necrotizing vasculitis in Churg-Strauss syndrome is morphologically indistinguishable from polyarteritis (medium-sized and small blood vessel lesions) and hypersensitivity angiitis (lesions affecting arterioles and venules). Tissue eosinophilia is variable, and extravascular granulomas may be difficult to find in biopsy or autopsy material (3, 48–51). The specificity of necrotizing granulomas in Churg-Strauss syndrome has also been questioned. Certainly, cases with the characteristic features of Wegener's granulomatosis as well as with asthma and eosinophilia have been described (71–74), lending support to the contention that Churg-Strauss syndrome may be a more benign variant of Wegener's granulomatosis, and both should be included under the generic term of *pathergic granulomatosis* (74–76). There is certainly no a priori reason that Wegener's granulomatosis cannot occur in an asthmatic patient. However, asthma is a central feature of Churg-Strauss syndrome and may precede the development of systemic vasculitis by up to 30 years (51), but it is unusual in Wegener's granulomatosis (77).

Of interest, recent studies of lymphocyte subpopulations in Wegener's granulomatosis (78) and in Churg-Strauss syndromes (79) both show a low proportion of suppressor/cytotoxic (T8+) cells and a high helper/suppressor ratio, suggesting that cellular rather than humoral-immune mechanisms predominate in both forms of pulmonary vasculitis. But, as Churg has contended, although eosinophilia >20% is commonplace in allergic granulomatosis and angiitis, it seldom occurs in Wegener's granulomatosis, and the two also differ in the anatomic locations of

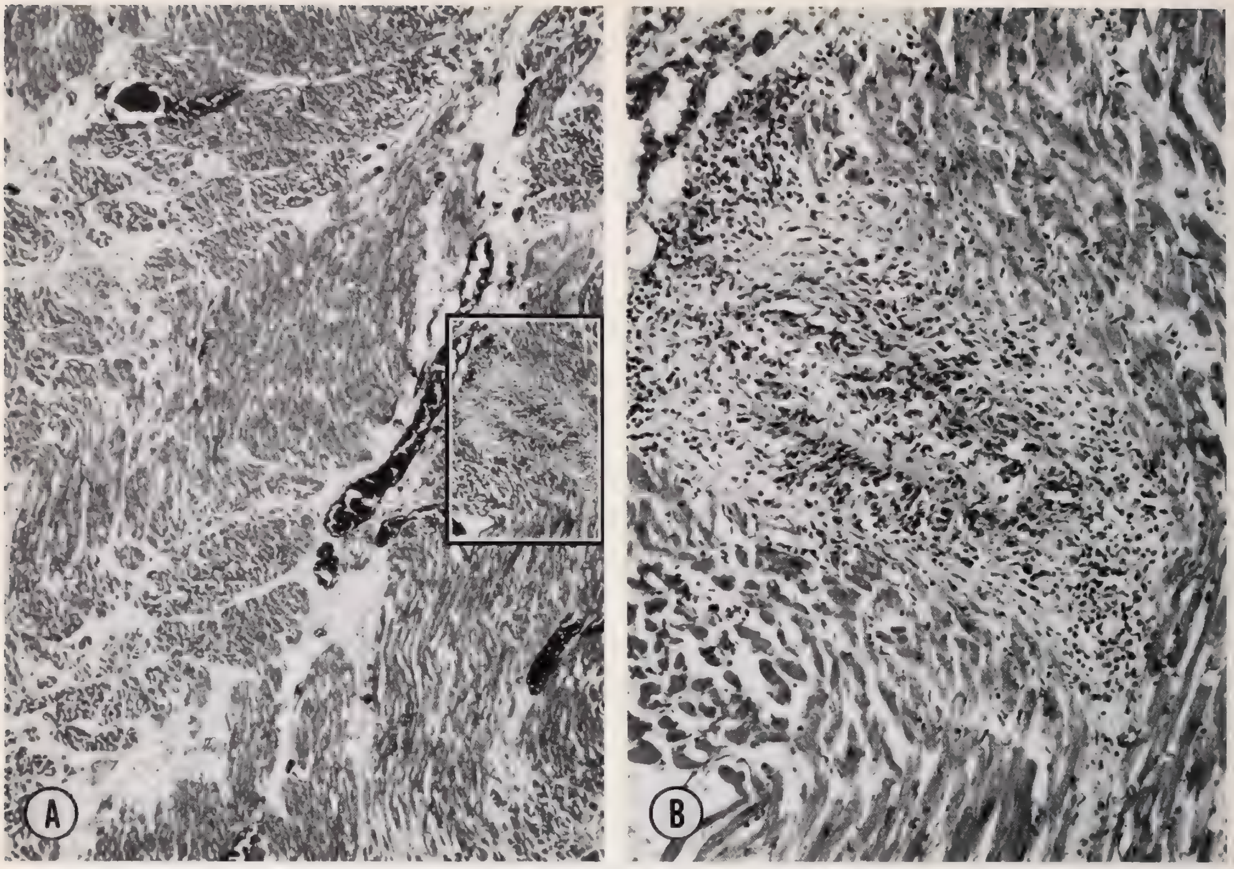

Fig. 7. (A) Necrotizing granuloma of myocardium in Churg-Strauss syndrome. (B), Close-up view of boxed area in A. Granuloma is reminiscent of myocardial lesion in rheumatoid arthritis. (Hematoxylin and eosin, A ×40; B ×160.)

lesions and frequency of renal involvement (49). As long as the cause of neither is known, it seems proper to separate Wegener's granulomatosis from Churg-Strauss syndrome (49, 80).

Clinical and Morphologic Distinctiveness. Nevertheless, there appears to be an apparent lack of familiarity with and therefore a reluctance to diagnose Churg-Strauss syndrome among necrotizing vasculitides by clinicians and pathologists alike. In an ongoing multicenter cooperative study of classification of vasculitis sponsored by the American Rheumatism Association, Churg-Strauss syndrome is the least common entity among the eight major types of vasculitis (Fig. 1). Just as the pattern of disease is sufficiently distinctive to justify the recognition of Churg-Strauss syndrome on clinical grounds (57), so is the morphologic approach to the diagnosis of this disease. This conclusion is based on a thorough review of all available pathologic materials of 54 patients, most of whom were subjects of previous reports from our institution (51, 61, 62).

There was a preponderance of males in this group of 54 patients: 37 males and 17 females, and their ages ranged from 3.5 to 69 years. All 54 patients fulfilled the criteria of Churg-Strauss syndrome, as proposed by Lanham and associates (57): asthma or a history of allergy, peak peripheral blood eosinophil count exceeding 1,500/μl, and systemic vasculitis involving two or more extrapulmonary organs. Necrotizing extravascular granulomas were observed in 39 (72%) patients.

Each of the major histopathologic features of Churg-Strauss syndrome may occur in isolation or may coexist, and they may be sparse or widely distributed. Necrotizing granulomas and vasculitis (Fig. 2) may be observed in a bronchial biopsy of an asthmatic who has roentgenographic evidence of diffuse pulmonary infiltrates. Extrapulmonary necrotizing granulomas and vasculitis are found most often in the spleen (Fig. 3) and gastrointestinal tract (Fig. 4), and less frequently in the kidneys (Fig. 5), lower urinary tract (Fig. 6), heart (Fig. 7), and muscle biopsy (Fig. 8). About half the patients had cutaneous and subcutaneous extravascular necrotizing granulomas (Fig. 9), which, as noted above (Table 2), also occur in many conditions other than Churg-Strauss syndrome.

Conclusion

A rational classification of vasculitis is not

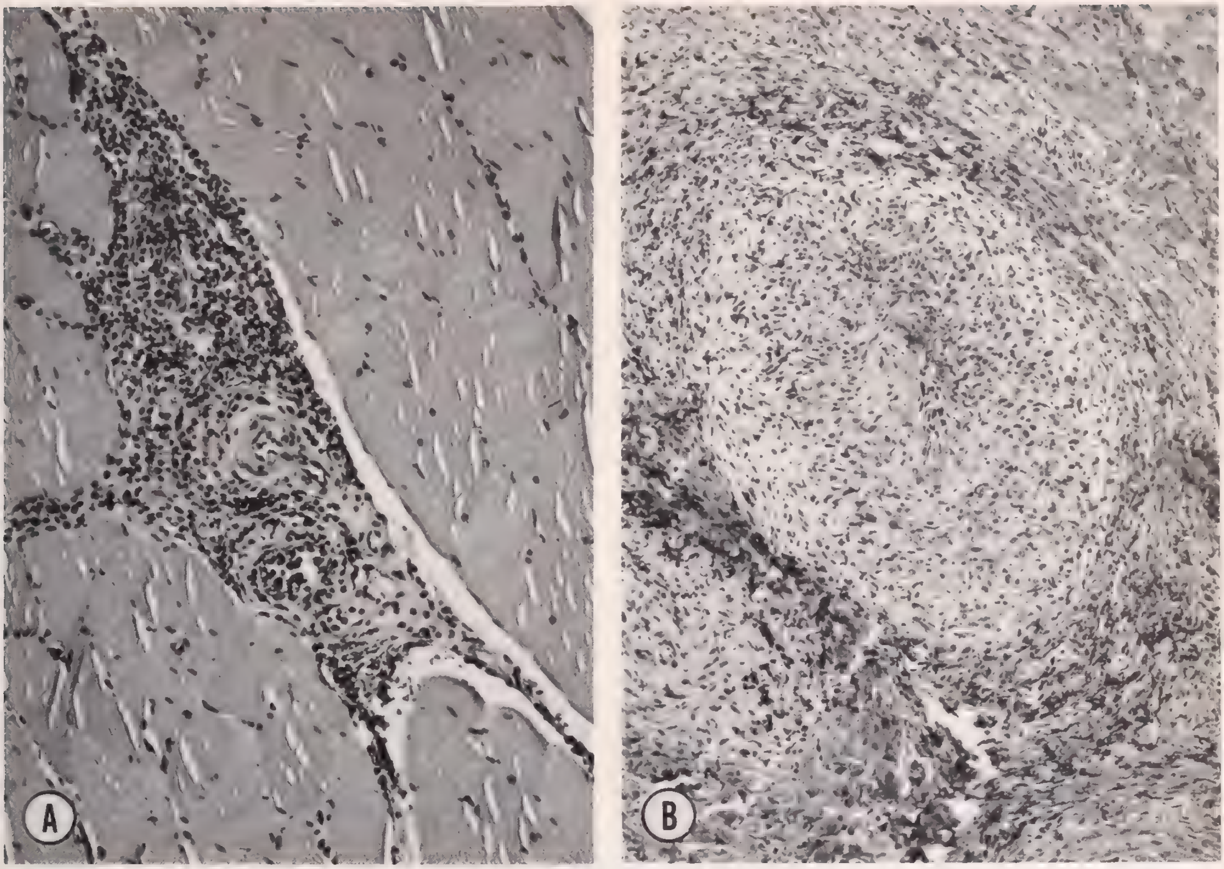

Fig. 8. Muscle biospy findings in Churg-Strauss syndrome. (A) Vasculitis with intense eosinophilic cell infiltrate; (B) extravascular granuloma with necrotic center surrounded by epithelioid cells. (Hematoxylin and eosin, × 160.)

possible until more is known about the etiology and pathogenesis of its different forms. A practical scheme of morphologic classification of vasculitis, based on the predominant types of blood vessels involved (Table 1), provides useful guidelines to clinical decision making, diagnosis, and management of patients with vasculitis.

Churg-Strauss syndrome is a legitimate member of the expanding clinical spectrum of systemic vasculitis. It is an "overlap" entity that

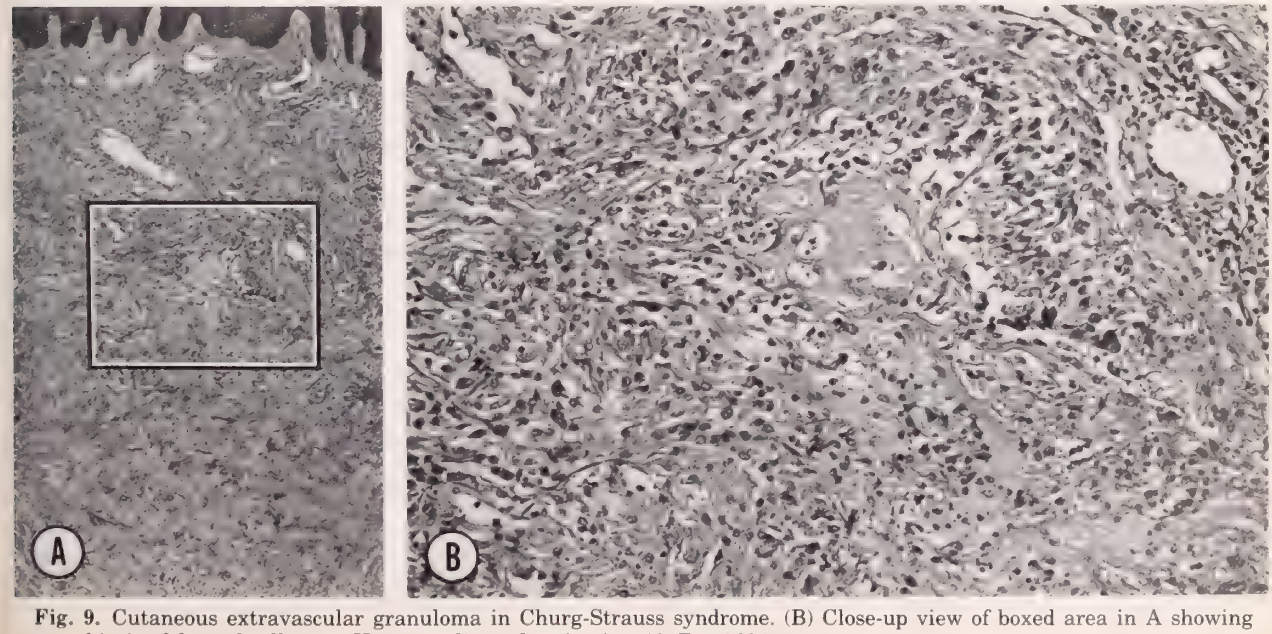

Fig. 9. Cutaneous extravascular granuloma in Churg-Strauss syndrome. (B) Close-up view of boxed area in A showing necrobiosis of dermal collagen. (Hematoxylin and eosin, A ×40; B ×160.)

is linked to polyarteritis nodosa on the one end and to Wegener's granulomatosis at the other end of the spectrum of granulomatous-vascular syndromes. From the prognostic and therapeutic standpoints, the separation of Churg-Strauss syndrome from other necrotizing systemic vasculitis is valuable. Whereas Wegener's granulomatosis and to a lesser extent polyarteritis often require treatment with cytotoxic agents, corticosteroids are effective in Churg-Strauss syndrome, according to the data in the literature.

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Therapeutic Approach to the Vasculitic Syndromes

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Vasculitis is an inflammation and necrosis of blood vessels leading ultimately to ischemia of the organs supplied by these vessels. Virtually any type of blood vessel and any organ system can be involved. Over the past 15 years much has been learned about the immunopathogenesis and treatment of these disorders. Although a range of heterogeneous syndromes are identified in the broad category of vasculitis (Table 1) (1), this article focuses only on the classification and treatment of the polyarteritis nodosa group of systemic necrotizing vasculitis, since this group of disorders is one of the most serious and invariably leads to irreversible organ system dysfunction and/or death if left untreated.

Immunopathogenic Mechanisms of Vasculitis

It is felt that most of the vasculitic syndromes are associated with the deposition of immune complexes in the blood vessel wall (2). Studies of the vasculitis associated with serum sickness and hepatitis B antigenemia have been helpful in our understanding of the immunopathogenesis of the vasculitic syndromes as a whole (2, 3). Briefly, it is postulated that circulating immune complexes are deposited in vessel walls. The deposition of complexes is probably enhanced by the increase in vascular permeability resulting from release of vasoactive amines from platelets and IgE-triggered basophils (4, 5). Complement-activated and complement-derived chemotactic factors cause infiltration of the vessels by polymorphonuclear leukocytes leading to damage of the vessel wall. In addition, granuloma formation can be caused by lymphokines released from either sensitized lymphocytes triggered by circulating antigen or from macrophages following ingestion of immune complexes (2). These theories do not explain adequately the heterogeneity of vasculitic syndromes associated with a single antigen such as hepatitis B. In addition, apart from hepatitis-B-associated vasculitis, in few vasculitic syndromes have specific antigens been identified in the immune complexes isolated from patients. Nonetheless this framework has provided a rational approach to therapy discussed in detail under the heading "Treatment."

Polyarteritis Nodosa Group of Systemic Necrotizing Vasculitis

Clinical and Pathologic Features. The polyarteritis nodosa group of systemic necrotizing vasculitis consists of three distinct vasculitic syndromes, all initially appearing as a systemic illness involving multiple organ systems. Signs and symptoms may be nonspecific, such as fever, malaise, weight loss, arthralgias, myalgias, and abdominal pain. Invariably, the patients have an elevated erythrocyte sedimentation rate. In the setting of a vague systemic illness with no apparent etiology and with an elevated erythrocyte sedimentation rate, one must maintain a high level of suspicion for vasculitis and search carefully for signs of specific organ system involvement. Vasculitic syndromes are generally diagnosed by demonstrating histopathologic evidence of vasculitis. A diagnosis of vasculitis cannot be made on clinical grounds alone. Occasionally, in the appropriate clinical setting, a diagnosis of

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polyarteritis nodosa or the polyangiitis overlap syndrome can be made on the basis of a positive visceral angiogram.

Classic polyarteritis nodosa is a necrotizing vasculitis involving small- and medium-sized muscular arteries (2, 6–9). Multiple organ systems may be involved, but the lung and spleen are usually spared (Table 2) (2, 10). A characteristic feature of classic polyarteritis nodosa is the finding of aneurysmal dilatations in medium-sized arteries which can be seen in the renal, hepatic, and visceral vasculature by angiography (Fig. 1) (10, 12–14).

Churg-Strauss syndrome, or *allergic angiitis and granulomatosis,* resembles classic polyarteritis nodosa in that it is a severe systemic vasculitis with multiple organ systems involved (Table 3). Distinct features of Churg-Strauss syndrome are its association with asthma, eosinophilia, and granuloma formation (2, 15). None of these features is associated with classic polyarteritis nodosa. In this regard, patients with Churg-Strauss syndrome have inflammation of capillaries and venules in addition to the typical necrotizing vasculitis seen in small- and medium-sized arteries in classic polyarteritis nodosa. Pulmonary involvement is common in Churg-Strauss syndrome.

The term *polyangiitis overlap syndrome* refers to systemic vasculitis in patients who cannot be classified neatly as having one of the well-defined vasculitic syndromes (2, 16). Many of these patients have syndromes with overlapping features of several distinct vasculitides (Table 4). The clinical features in patients with the polyangiitis overlap syndrome (Table 5) are similar to those noted in Tables 2 and 3. It is important to recognize the existence of the polyangiitis overlap syndrome since it is a systemic vasculitis syndrome with the potential for irreversible organ system damage if not adequately treated.

Series of 22 Patients. We have followed up 22 patients at the National Institutes of Health who fall into the polyarteritis nodosa group of systemic necrotizing vasculitis (Table 6). Of the 10 patients who have classic polyarteritis nodosa, 6 are hepatitis B surface-antigen positive.

In addition to these 22 patients, one patient with well-documented Churg-Strauss vasculitis was followed up but not included in this series. She was referred to us for therapy. Three months after starting therapy, the vasculitis was in remission but the patient developed metastatic malignant melanoma. She died 5 months after she was referred and at autopsy had no evidence of active vasculitis. Several other patients who fall into the polyarteritis nodosa group of systemic necrotizing vasculitis have also been followed up, but complete follow-up data are not available and they are not included in this series.

TABLE 1

Classification of the Vasculitides by Clinical and Pathologic Criteria

Systemic necrotizing vasculitis (polyarteritis nodosa group)
Classic polyarteritis nodosa
Allergic angiitis and granulomatosis
"Polyangiitis overlap syndrome"

Hypersensitivity vasculitis
Henoch-Schönlein purpura
Serum sickness and sicknesslike reactions
Other drug-related vasculitides
Infectious disease association
Neoplasm (mostly lymphoid) association
Connective tissue disease association
Other underlying disease association
Congenital deficiencies of complement system
Erythema elevatum diutinum

Wegener's granulomatosis

Giant cell arteritides
Cranial or temporal arteritis
Takayasu's arteritis

Other vasculitic syndromes
Mucocutaneous lymph node syndrome (Kawasaki's disease)
Behçet's disease
Thromboangiitis obliterans (Buerger's disease)
Miscellaneous vasculitis

Adapted from ref 1.

Churg-Strauss syndrome, which is rare, occurred in 2/22 (9%) of our patients with the polyarteritis nodosa group of systemic necrotizing vasculitis. However, three patients in the polyangiitis overlap syndrome group had syndromes which overlapped classic polyarteritis nodosa and Churg-Strauss syndrome. Each had asthma, eosinophilia, pulmonary infiltrates, and multiple aneurysms in the visceral vasculature. The literature is inconsistent in its categorization of patients with overlapping syndromes, sometimes including them with classic polyarteritis nodosa patients and sometimes with Churg-Strauss syndrome patients (17, 18).

Treatment. A general approach to the treatment of vasculitis syndromes, including the rationale for therapy, is as follows:

- Removal of offending antigen: withdrawal of drug or environmental agent, treatment of infection.
- Prevention of deposition of circulating immune complexes in vascular bed: plasmapheresis.

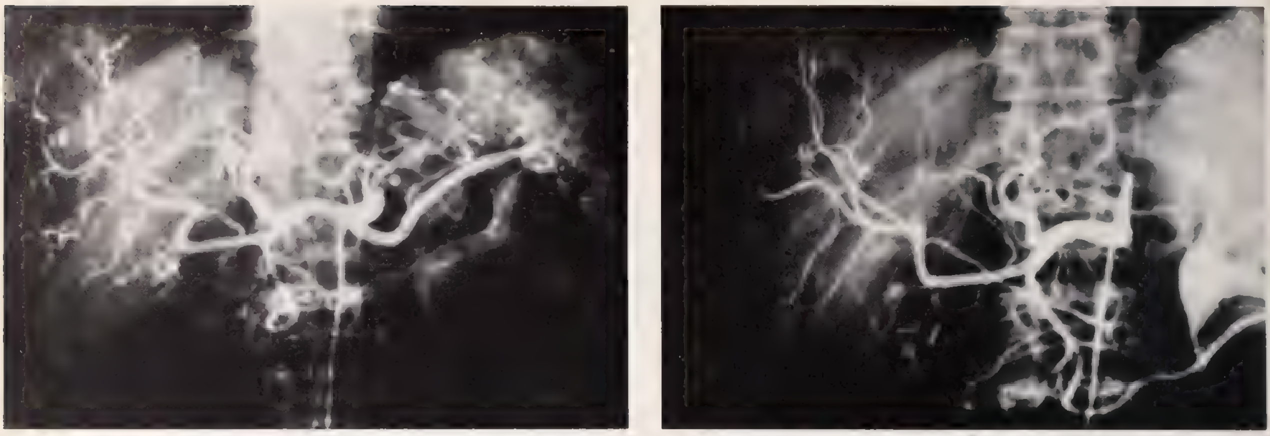

Fig. 1. Selective celiac arteriogram demonstrates large hepatic arteries and multiple aneurysms (**left**) throughout liver. One year after therapy (**right**), hepatic arteries are smaller and all aneurysms have disappeared. Reprinted from ref. 11.

- Suppression of inflammatory response resulting from immune complex deposition: antiinflammatory agents such as corticosteroids.
- Modulation of immune response: corticosteroids, cytotoxic agents, antilymphocyte serum, lymphapheresis.

TABLE 2

Clinical Parameters in Patients with Classic Polyarteritis Nodosa

Clinical parameter	Value
General	
Age (mean)	45 years
Sex (M:F)	2.5:1
Fever	71%
Weight loss	54%
Organ system involvement	
Kidney, renal	70%
Musculoskeletal system	64%
Arthritis/arthralgia	53%
Myalgias	31%
Hypertension	54%
Peripheral neuropathy	51%
Gastrointestinal tract	44%
Abdominal pain	43%
Nausea/vomiting	40%
Cholecystitis	17%
Bleeding	6%
Bowel perforation	5%
Bowel infarction	1.4%
Skin	43%
Rash/purpura	30%
Nodules	15%
Livedo reticularis	4%
Cardiac	36%
Congestive heart failure	12%
Myocardial infarction	6%
Pericarditis	4%
Central nervous system	23%
Cerebral vascular accident	11%
Altered mental status	10%
Seizure	4%

Adapted from ref. 10.

Combination therapy with corticosteroids and cyclophosphamide has been shown to be extremely effective in treating systemic vasculitis (2, 10, 19, 20). Prior to the use of corticosteroids, the five-year survival in untreated patients with these disorders was 13% (21). The five-year survival increased to 48% following therapy with corticosteroids (21), and our data show a 90% five-year survival following treatment with cyclophosphamide and corticosteroids (Table 6).

Our protocol (20) for combined therapy with cyclophosphamide and prednisone is as follows:

Cyclophosphamide component: Cyclophosphamide, 2 mg/kg/day orally; adjust dosage such that the white blood cell count remains above 3,000–3,500/mm^3 (neutrophil count of 1,000–1,500/mm^3). Continue therapy with frequent downward adjustments of dosage so that severe neutropenia does not occur. Continue therapy for 1 year following induction of complete remission, at which point dose is tapered by 25-mg decrements every 2 months until discontinued.

Prednisone component: Prednisone, 1 mg/kg/day in 3–4 divided doses for 7–10 days; consolidate to single morning dose by 2–3 weeks; continue single morning dose until 1-month total steroid treatment is reached. Convert to alternate-day prednisone over the second month; maintain alternate-day regimen over third month, followed by gradual tapering of alternate dose over the next 3–6 months.

Corticosteroids have been useful in the therapy of virtually all of the vasculitic syndromes (2, 10, 20). They have both antiinflammatory and immunosuppressive effects and appear to act through multiple mechanisms (22). These effects are seen immediately, and corticosteroids are especially important in the initial treatment of vasculitis, since cytotoxic agents often take 2–3 weeks to induce maximal immunosuppression. Prednisone is the corticosteroid of choice in treating vasculitis, since it has a relatively short half-life and therefore has less of a suppressive effect on the hypothalamic-pituitary-adrenal axis than other longer-acting corticosteroids such as dexamethasone.

Prednisone is initially given at a dose of 1 mg/kg/day in three or four divided doses. This regimen provides maximal antiinflammatory and immunosuppressive effects; however, it also has a high incidence of side effects if administered long term. The consolidated daily prednisone, usually combined with cyclophosphamide, is continued for about one month, when a clear response should be achieved. Over the second month of therapy, the prednisone dose is gradually tapered to an alternate-day regimen, which is important, since the toxic side effects of prednisone are markedly reduced when the drug is given on alternate days (23). A typical patient is tapered from 60 mg of prednisone/day to 60 mg of prednisone every other day over a month by initially tapering at 10 mg/cycle (60 mg, 50 mg, 60 mg, 40 mg, 60 mg, 30 mg) until the dose of 30 mg on the ultimate "off" day is reached. At this point, the alternate-day dose is decreased by 5 mg/cycle until a dose of 10 mg is reached, and then by 2.5 mg/cycle until the only dose is 60 mg every other day. This dose is maintained for another month and then gradually tapered as tolerated. Optimally, patients require only 6–8 months of prednisone therapy when used in combination with cyclophosphamide. If the patient remains in remission, the prednisone is gradually tapered.

Cyclophosphamide is an alkylating agent which causes immunosuppression by inhibiting both T- and B-lymphocyte function (24). The drug is activated in the liver and excreted through the kidney in the form of active metabolites. The immunosuppressive effects of long-term administration of cyclophosphamide in humans (24) are as follows:

- Absolute lymphocytopenia of both T and B cells; early preferential depletion of B cells.
- Significant suppression of in vitro lymphocyte blastogenic responses to specific antigenic stimuli; only mild suppression of responses to mitogenic stimuli.
- Suppression of antibody response and cutaneous delayed hypersensitivity to a new antigen; relative sparing of established cutaneous delayed hypersensitivity.

TABLE 3

Clinical Parameters in Allergic Angiitis and Granulomatosis

Category	Results
General	
Age (mean)	44 yrs
Sex (M:F)	1.3:1
Fever	Majority of patients
Duration (mean) of pulmonary symptoms prior to systemic symptoms	2 yrs
Organ system involvement	
Pulmonary	96%
Infiltrate on chest x ray	93%
Wheezing	82%
Cutaneous	67%
Purpura	37%
Nodule	35%
Peripheral neuropathy	63%
Hypertension	54%
Gastrointestinal	42%
Cardiac	38%
Renal	38%
Lower urinary tract	10%
Arthritis/arthralgia	21%

Adapted from reference 10.

TABLE 4

Syndromes Seen in the Polyangiitis Overlap Syndrome

Overlapping syndromes	Patients No.	Patients %
Systemic necrotizing vasculitis, unclassified	2	20
Polyarteritis nodosa and Churg-Strauss	3	30
Temporal arteritis and polyarteritis nodosa	1	10
Takayasu's arteritis and polyarteritis nodosa	1	10
Polyarteritis nodosa and cutaneous vasculitis	1	10
Henoch-Schönlein purpura and polyarteritis nodosa	1	10
Giant cell arteritis and systemic granulomatous vasculitis	1	10

- Reduction of elevated serum immunoglobulin levels as well as occurrence of hypogammaglobulinemia in patients treated for extended periods (years).
- Selective suppression of in vitro B-cell function with diminution of increased spontaneous immunoglobulin production of individual B cells as well as suppression of mitogen-induced immunoglobulin production.

Cyclophosphamide is given at a dose of 2 mg/kg/day as a single oral dose, but can be given intravenously, if necessary. In patients with fulminant, life-threatening disease, the dose is 4

TABLE 5

Clinical Parameters in Patients with Polyangiitis Overlap Syndrome

Parameter	Results
Number of patients	10
Age (mean)	26 years
Sex (M/F)	1.5/1
Organ system involvement	
Skin	90%
Lung	50%
Musculoskeletal	40%
Kidney	30%
Gastrointestinal	30%
Peripheral nervous system	30%
Heart	10%
Other	
Aortic arch	10%
Temporal artery	10%

mg/kg/day for the first 3 days, which is then decreased to the optimal dose of 2 mg/kg/day. The goal of therapy with cyclophosphamide is to suppress the underlying disease activity without causing significant neutropenia. The maximal immunosuppressive effects of the drug are not seen for at least 2 weeks. This delay in the onset of immunosuppression is one reason for the important role of prednisone in the initial treatment of vasculitis.

We have found that if the peripheral white blood cell count is maintained above 3,500 cells/mm^3, which results in a neutrophil count of greater than 1,000 cells/mm^3, patients do not develop opportunistic infections. However, in patients maintained on long-term cyclophosphamide therapy there does appear to be an increased incidence of herpes zoster which is unrelated to the peripheral white blood cell count (25). Since the association of neutropenia with an increased incidence of opportunistic infections is well documented, the treating physician must monitor white blood cell counts frequently and adjust the cyclophosphamide dose accordingly (26). Because the effects of cyclophosphamide are not seen for several days, dosage adjustments may need to be made when the white blood cell count is beginning to decrease (Fig. 2). If the dose is not adjusted until the white blood cell count has already decreased to 3,500 mm^3, the count will continue to fall, resulting in severe neutropenia (Fig. 2).

TABLE 6

Features of 22 Patients with the Polyarteritis Group of Systemic Necrotizing Vasculitis Followed Up at National Institutes of Health

	Classic polyarteritis nodosa	Churg-Strauss syndrome	Polyangiitis overlap syndrome
No. of patients	10	2	10
Male	7	1	6
Female	3	1	4
Mean age at onset (years)	48	32	26
Mean duration of follow-up (months)	63	95	64
No. in remission (%)	8*	2 (100%)	10 (100%)
On therapy	0	1	1
Off therapy	8	1	9

* Two patients died in remission while on therapy.

Daily cyclophosphamide is continued for 1 year following the induction of complete remission and then tapered by 25-mg decrements every 1–2 months with careful monitoring for any signs of disease activity. Although not used in a controlled study, bolus intravenous cyclophosphamide in our anecdotal experience is relatively ineffective in inducing remission in patients with systemic vasculitis (20).

The combination of prednisone and cyclophosphamide has been extremely successful in treating patients in the polyarteritis nodosa group of systemic necrotizing vasculitis (Fig. 1) and provides better control of disease activity than either agent alone. In general, patients with severe necrotizing vasculitis are started on the therapeutic protocol outline above. We have found that in patients with visceral involvement, prednisone alone is generally not effective in inducing and sustaining long-term remission (19).

Of the 22 patients (Table 6) we followed up for a mean of 5.5 years, 18 were treated with combination prednisone-cyclophosphamide therapy, 2 with prednisone only, 1 with cyclophosphamide alone, and 1 with a combination of prednisone and azathioprine. These patients were referred to us at different stages of disease activity and therapy. Most were referred because their disease activity could not be controlled with prednisone alone. However, some were referred for a second opinion with good control of their disease activity; these patients were followed up without change in their therapy. Of our 22 patients, 20 (91%) are alive and in remission; mean duration of remission is 5.1 years. Two patients died while in remission on therapy. Eighteen (82%) patients remain in remission off medication (mean duration off medication, 3.4 years). Two patients have relapsed; both had the polyangiitis overlap syndrome. One patient relapsed 13 months after medications were discontinued, was successfully retreated with cyclophosphamide and prednisone, and has been in remission off medications for 21 months. The other patient relapsed within 1 month of discontinuing prednisone therapy. This patient had previously developed toxic side effects on both cyclophosphamide and azathioprine and was successfully retreated with prednisone alone. She has been in remission for 44 months but remains on low-dose, alternate-day prednisone secondary to severe asthma. In our patients, the mean duration of corticosteroid therapy is 2.6 years and the mean duration of cyclophosphamide therapy is 2.4 years.

Azathioprine, a purine analog, has been used in the treatment of both systemic necrotizing vasculitis (19) and Wegener's granulomatosis (27, 28). Cyclophosphamide is clearly more effective than azathioprine in inducing a remission in the vasculitic syndromes, but azathioprine can be effective in maintaining remission (19, 20, 27), and is also an alternative in patients who cannot tolerate cyclophosphamide. Azathioprine is administered at a dose of 2 mg/kg/day. As with cyclophosphamide, dosage adjustments are made according to the degree of myelosuppression. The immunosuppression produced by azathioprine is distinct from that of cyclophosphamide in that opportunistic infections can occur even in the presence of a normal peripheral white blood cell count (24). With cyclophosphamide therapy, the peripheral white blood cell count accurately reflects the degree of immunosuppression. Hepatic toxicity can be produced by azathioprine.

Chlorambucil, an alkylating agent, has been used successfully in the treatment of limited forms of Wegener's granulamotosis (29, 30). However, it is less effective than cyclophosphamide and has not been useful in treating the renal disease associated with Wegener's granulomatosis (30). An advantage of chlorambucil is that it does not cause hemorrhagic cystitis, which is a major and frequent complication of cyclophosphamide therapy, often necessitating discontinuation of the drug. Chlorambucil therapy has not been extensively studied in the polyarteritis nodosa group of systemic necrotizing vasculitis. Therefore, it should be used cautiously in patients who cannot receive the more standard cytotoxic agents such as cyclophosphamide and azathioprine. For use in patients with vasculitis, chlorambucil is given at a dose of 0.1–0.2 mg/kg/day orally. As with cyclophosphamide, the dose is adjusted to maintain a peripheral white blood cell count of greater than 3,500 cells/mm^3. Other side effects include gastrointestinal discomfort, hepatotoxicity, dermatitis, and infertility (24). There appears to be significant incidence of oncogenesis, especially leukemia, in patients receiving chlorambucil (31, 32).

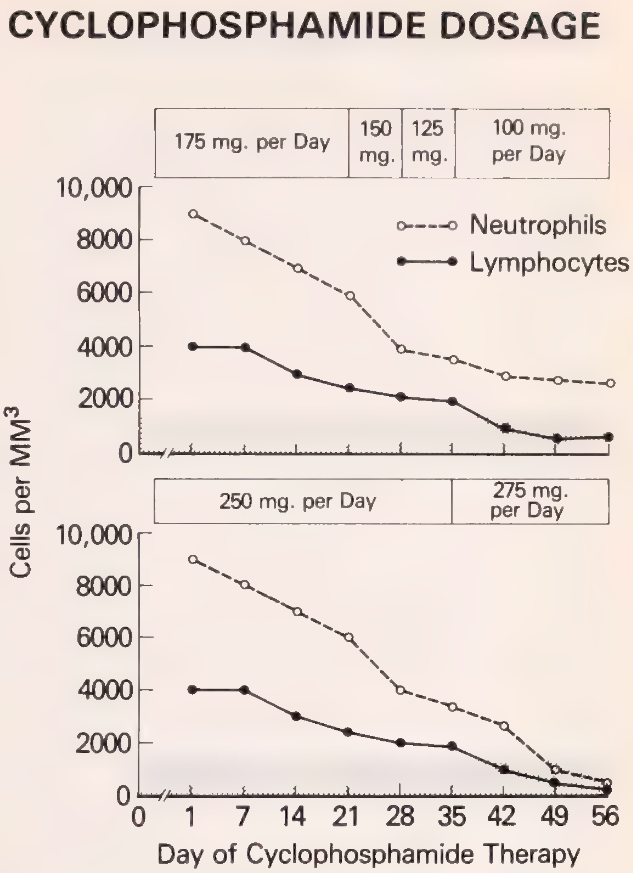

Fig. 2. Neutrophil and lymphocyte cell counts in relation to dose of cyclophosphasmide are plotted over time. **Top** Patient on an optimally managed immunosuppressive regimen. Dose of cyclophosphamide is reduced as leukocyte count drops, so that suitable level of immunosuppression is obtained without suppression of neutrophil count below 1,500 cells/mm^3. **Bottom** Patient who has not received optimal management of cytoxic regimen. Instead of a reduction in dose of cyclophosphamide as leukocyte count dropped, dose was increased. At this point, neutrophil count showed selectively greater reduction than lymphocyte count, which had only minimal decrease compared with neutrophil count. Patient subsequently developed neutropenia with a total neutrophil count below 1,500 cells/mm^3 and was at increased risk of developing opportunistic infection. Reprinted from ref. 10.

Plasmapheresis, the removal of plasma without removal of cellular elements, has been used to remove pathogenic circulating agents such an antibodies and immune complexes. It has been reported to be an effective therapeutic modality in treating Goodpasture's syndrome (33), systemic lupus erythematosus (34), and rheumatoid arthritis (35). The rationale for using plasmapheresis in patients with vasculitis is to remove circulating immune complexes. We have found that although high levels of circulating im-

TABLE 7
Complications of Corticosteroid Therapy

Central nervous system
Pseudotumor cerebri
Psychiatric disorders
Musculoskeletal
Osteoporosis with spontaneous fractures
Aseptic necrosis of bone
Myopathy
Ocular
Glaucoma
Cataracts
Gastrointestinal
Peptic ulceration
Intestinal perforation
Pancreatitis
Cardiovascular and fluid balance
Hypertension
Sodium and fluid retention
Hypokalemic alkalosis
Hypersensitivity reactions
Urticaria
Anaphylaxis
Endocrinologic
Suppression of HPA axis
Growth failure
Secondary amenorrhea
Metabolic
Hyperglycemia and unmasking of genetic predisposition to diabetes mellitus
Nonketotic hyperosmolar states
Hyperlipidemia
Alterations of fat distribution (typical cushingoid appearance)
Fatty infiltration of liver
Drug interactions (decreased anticoagulant effect of ethyl biscoumacetate)
Fibroblast inhibition
Inhibition of wound healing
Subcutaneous tissue atrophy (striae, purpura, ecchymosis)
Suppression of host defenses
Immunosuppression, anergy
Effects on phagocyte kinetics and function
Increased incidence of infections

Adapted from ref. 20.

mune complexes correlate with active disease, many patients without high levels of circulating immune complexes have very active disease. However, there is evidence to suggest that certain therapeutic effects of plasmapheresis do not correlate with the removal of autoantibodies or immune complexes (36). Plasmapheresis is generally used in combination with immunosuppressive agents in patients with fulminant disease; hence the efficacy of plasmapheresis alone in the treatment of vasculitis is difficult to assess. Plasmapheresis is usually performed as a 3-liter plasma exchange three times a week for 2–6 weeks.

Cyclosporin A, a cyclic endecapeptide with potent immunosuppressive effects, has been used extensively in patients undergoing organ transplantation (37). It has a low degree of myelotoxicity and primarily affects T-lymphocyte function (37). It has been shown to inhibit production of interleukin 2 (T-cell growth factor) in T cells (38). Although cyclosporin A has not been used in the treatment of vasculitis, it is clearly an agent to be considered for use in the future. The major toxic side effect of the drug has been nephrotoxicity (37).

Toxic Side Effects and Complications of Therapy. Although the combination therapy described above for systemic vasculitis is generally effective, significant toxic side effects are associated with both prednisone and cyclophosphamide. In this regard, it is extremely important to establish a tissue diagnosis before instituting therapy in order to avoid inappropriate treatment of a nonvasculitic syndrome. Virtually all the side effects of corticosteroid therapy (Table 7) are minimized when the drug is administered as an alternate-day regimen (20, 23).

The complications of chronic cyclophosphamide therapy (20) are marrow suppression, particularly neutropenia, with resulting secondary infection; hemorrhagic cystitis; gonadal and ovarian dysfunction; alopecia; and oncogenesis.

The risk of opportunistic infections can be virtually eliminated if the peripheral white blood cell count is maintained above 3,500 cells/mm^3. Patients treated with cyclophosphamide have an increased incidence of herpes zoster which is unrelated to the peripheral white blood cell count (25). Although cutaneous dissemination of the virus has occurred, none of our patients has developed visceral disease. The patient is at greatest risk of opportunistic infection while on daily prednisone and cyclophosphamide. Therefore, one should make every effort to taper the prednisone to an alternate-day regimen according to the protocol for combination therapy described above, and carefully monitor the peripheral white blood cell count. Often, the peripheral white blood cell count decreases during the prednisone taper, requiring a decrease in the cyclophosphamide dose.

Hemorrhagic cystitis is a disturbing complication of cyclophosphamide therapy which often necessitates discontinuing the drug. The risk of hemorrhagic cystitis can be minimized by giving the drug in the morning and maintaining adequate hydration throughout the day. The drug should be discontinued at the first sign of cystitis, and cystoscopy should be performed. Recently, a drug called Mesna has been reported to prevent hemorrhagic cystitis in patients receiving cyclophosphamide (39, 40).

Gonadal dysfunction is a serious side effect of cyclophosphamide therapy. We suggest that males store semen prior to the onset of therapy, if possible. Sterility in women is related to the cumulative cyclophosphamide dose received. The total amount of cyclophosphamide administered daily at a dose of 1–2 mg/kg/day over a year of therapy invariably results in infertility in women. There is some evidence that suppressing ovarian function during therapy may result in preservation of ovarian function in women receiving chemotherapy for the treatment of neoplastic diseases (41–43). Therefore, we now administer oral contraceptives to women of childbearing age who are receiving long-term cyclophosphamide for systemic vasculitis. We have not yet accumulated sufficient data to establish the efficacy of this approach.

Summary

The polyarteritis nodosa group of systemic necrotizing vasculitis comprise a group of disorders with the potential for irreversible organ system damage, and untreated patients have a five-year survival rate of less than 15%. Combination prednisone and cyclophosphamide is effective therapy for these disorders, resulting in a five-year survival rate of 90%.

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Biopsies of the Lung and Bronchial Wall by Flexible Fiberoptic Bronchoscopy in 75 Patients with Sarcoidosis in Turkey

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As is generally known, the diagnosis of sarcoidosis can only be established with certainty by detection of the characteristic granulomata in tissue biopsies, together with, or without, a positive Kveim-Siltzbach skin test (1).

In cases of intrathoracic sarcoidosis without involvement of accessible superficial organs such as peripheral lymph nodes, skin, and parotid gland, or in geographic areas where the Kveim-Siltzbach skin test cannot be performed, or in cases with negative skin tests, histologic verification creates a difficult diagnostic problem. In such cases, methods such as scalene lymph node biopsy, thoracotomy, mediastinoscopy, rigid bronchoscopy, or percutaneous lung biopsies are generally applied.

In the application of these latter methods, the necessity of using general anesthesia and its inherent risk for the patient limits their extensive use.

It is felt now that transbronchoscopic lung and bronchial biopsies obtained by flexible fiberoptic bronchoscope (FFB) for the histopathological diagnosis of diffuse pulmonary diseases may also be used successfully in cases of intrathoracic sarcoidosis (2–6).

Patients, Materials and Methods

Transbronchoscopic biopsies obtained by FFB were performed in 75 patients with sarcoidosis during the years 1979–1983 in the Department of Internal Medicine of Cerrahpaşa Medical Faculty. In all of these patients the diagnosis had been established, along with compatible clinical and radiologic criteria, by one or more organ biopsies showing epitheloid granulomas without caseous centers, and/or positive Kveim-Siltzbach skin tests.

The 75 patients with sarcoidosis in whom transbronchoscopic biopsies were performed included 52 females and 23 males. The mean age was 41 ± 14 years in females and 34 ± 11 years in males. The youngest patient was 13 and the oldest was 71 years of age.

The Olympus BF B2 and BF 2 TR type flexible fiberoptic bronchoscopes were used, bronchoscopy being performed transnasally with the patient under local anesthesia and in a sitting position. Biopsies of the lung tissue just adjacent to the bronchus were taken by means of the Olympus type FB 19C forceps, without fluoroscopy, so-called transbronchial biopsy. Additional mucosal biopsies from subcarinal regions of small bronchi or from the walls of the larger bronchi were collected from some of the patients.

The term "transbronchoscopic biopsy" has been used to designate the transbronchial parenchymal and bronchial mucosa biopsies together. A minimum of 7 pieces (biopsies) were taken in order to increase the chance of finding the typical granulomas (2, 5). In 6 patients a self-healing pneumothorax appeared, as a complication of the procedure.

The Kveim-Siltzbach intradermal skin test was performed simultaneously with transbronchoscopic biopsies in 43 of the 75 patients. A suspension prepared by the late Dr. L. E. Siltzbach of The Mount Sinai Hospital, New York, NY was used in all of the Kveim tests (7).

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TABLE

Results of Transbronchoscopic Biopsies by Radiologic Stage in 75 Patients with Sarcoidosis

Radiologic stages	No. of patients	Results of transbronchial biopsies: Alveolar	Bronchial	Alveolar and bronchial	No. of positive biopsies	Ratio of positivity (%)
Normal (stage 0)	4	1	—	—	1	25
Bilateral hilar adenopathy (stage I)	24	8	4	1	13	54
Bilateral hilar adenopathy + pulmonary mottling (stage II)	28	13	8	2	23	82
Pulmonary mottling only (stage III)	19	8	6	2	16	84
TOTAL	75	30	18	5	53	70

Results

In 53 of the 75 patients, epithelioid histiocytic granulomas characteristic of sarcoidosis were found in the lung parenchyma or in bronchial mucosa, or in both simultaneously.

The typical granulomas were found only in the parenchyma in 30, only in the bronchial mucosa in 18, and in both parenchyma and in bronchial mucosa in 5 patients (Table).

During bronchoscopy 13 of the 75 cases (17%) had yellowish-pink nodules, ranging from pinpoint to 2 mm in diameter, visualized grossly. On the bronchial mucosal surface, biopsies were taken from these nodules as well as from lung parenchyma. In these 13 cases, granulomas were detected in the bronchial mucosa alone in 10 cases, and observed in lung parenchyma alone in 3 cases.

The results of transbronchoscopic biopsies by radiologic stages are shown in Table. In 13 of 24 cases (54%) with bilateral hilar lymphadenopathy without visible parenchymal mottling (Stage I), transbronchoscopic biopsy revealed the typical granulomas. Granulomas were found only in the lung parenchyma in 8, only in bronchial mucosa in 4, and in both sites in 1 case.

In 23 of 28 cases (82%) with bilateral hilar adenopathy together with parenchymal mottling (Stage II), transbronchoscopic biopsies revealed granulomas. The typical lesions were found in alveoli in 13, only in bronchial mucosa in 8, and in both sites in 2 cases.

In 16 of 19 cases (84%) with parenchymal mottling and without lymphadenopathy (Stage III), biopsies showed typical lesions. Granulomatous lesions were in the alveoli in 8, only in the bronchial mucosa in 6, and in both sites in 2 cases.

In 1 of 4 cases with extrapulmonary involvement without radiologic findings, typical granulomata were found in the parenchyma.

Positive Kveim-Siltzbach skin tests were obtained in 33 of the 43 (77%) patients in whom this test was applied. In 22 of the 43, both transbronchoscopic biopsy and Kveim-Siltzbach tests were positive; in 6, transbronchoscopic biopsy was positive, whereas the Kveim-Siltzbach test was negative; in 11, the Kveim-Siltzbach test was positive whereas the transbronchial biopsy was negative; and in 4, both were negative. Thus, in 39 of 43 patients, simultaneously applied transbronchoscopic biopsy and Kveim-Siltzbach testing resulted in a definite histopathologic diagnosis in 90% of instances.

Discussion

Studies done during the last 10 years have shown that bronchial wall and lung parenchymal biopsies obtained by means of FFB are of valuable assistance in the histopathological diagnosis of sarcoidosis.

Sarcoidosis granulomas have been detected by transbronchial biopsy in 20 out of 25 (80%) cases by Teirstein et al (6). Koerner et al (3) found sarcoidosis granulomas in parenchymal biopsies of 21 of 26 (80%) cases, and Mitchell et al found the typical granuloma of sarcoidosis in 37 of 42 (88%) transbronchial lung biopsies and in 17 of 22 (77%) bronchial mucosa biopsies (4).

It has also been shown that biopsies of bronchial mucosa obtained by rigid bronchoscope are helpful in the histologic diagnosis of sarcoidosis. Lemoine et al (8) found typical granulomas in the bronchial mucosa biopsies in 60% of 180 cases and Friedman et al (9) in 22 of 35 cases by means of the rigid bronchoscope.

FFB, because of the facility of its application, its ability to reach the lung parenchyma, and a higher rate of positive results, has virtually replaced the rigid bronchoscope for diagnosing interstitial lung disease (10).

Our finding of typical sarcoid granulomas in 53 of a series of 75 cases (70%) has confirmed previous results. The fact that characteristic granulomata were found in 54% of 24 cases with bilateral hilar adenopathy in the absence of radiologic parenchymal findings (Stage I) emphasizes the fact that granulomas of sarcoidosis may be present without radiologic signs. The rate of positive biopsies increases in the cases of discernible pulmonary mottling with or without bilateral hilar adenopathy. Lesser frequency (54%) of positive FFB in radiologic stage I sarcoidosis could be complemented by use of the Kveim-Siltzbach test with 77% positive results, which is also supported by the results obtained by Gilman, Koerner, Mitchell, Roethe, and Teirstein (2–6).

There is also suggestion that transbronchial biopsy in cases of extrapulmonary sarcoidosis without radiologic thoracic findings will reveal typical granulomas in the parenchyma, since 1 of 4 such cases was positive for granuloma. All of these findings suggest that the lung may be involved at all times in any form of sarcoidosis.

Granulomas found in bronchoscopic biopsies may not always prove the presence of sarcoidosis definitively, since the same type of granulomata can also be found in tuberculosis, in extrinsic allergic alveolitis, and in other conditions. Therefore, any positive results suggesting sarcoidosis should be evaluated only by taking into consideration the presence of characteristic clinical and radiologic signs of sarcoidosis. If the diagnosis still remains uncertain, Kveim-Siltzbach skin testing should be applied or biopsies taken of other organs, such as muscle, lacrimal gland, oral mucosa. In our study, the application of transbronchoscopic biopsy together with the Kveim-Siltzbach test proved to be sufficient for a definitive diagnosis in 90% of 43 cases in which both were performed.

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Sex, Age, and the Pancreas

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Abstract

The exocrine pancreatic secretory response to an intravenous bolus of secretin (1.0 U/kg) was studied in 2 groups of healthy men and women each stratified by age (below and above the age of 45). The secretory pattern—electrolyte and enzyme secretion of eight sequential 10-minute collections—and cumulative values for each parameter were studied. In the male group, no significant changes were observed, except for an increase of amylase and lipase secretion in the older group with respect to the younger one. In the female group, on the other hand, the hydrelatic pattern showed a decline of flow and of bicarbonate concentration and output in women over 45 years. When comparing the young male and the young female subjects, no significant secretory differences were observed. On the other hand, comparison of men and women over 45 disclosed a significant loss of bicarbonate concentration and output in women. In women over 45 years, moreover, the 80-min cumulative bicarbonate output and lipase output were significantly below those parameters in men. These changes are interpreted as resulting from changes in hormonal balances that occur at the menopause-andropause. Declining estrogen activity would lead to a loss of estrogen receptor activation, and this loss of estrogen tone would diminish the facilitating or potentiating action on secretin-induced hydrelatic and ecbolic response of the pancreon.

The aim of this work was to elucidate the influence of age and sex on secretin-induced pancreatic secretion. In a previous paper (1) it was shown, in contradistinction to the findings of Rosenberg et al (2), that the hydrelatic pancreatic response is significantly higher in men than in women. These observations did not include stratification for age.

This study was undertaken as a follow-up to two recent findings. The first (unpublished observations) showed that estradiol administered for less than one month to menopausal women resulted in an increase of secretin-induced pancreatic flow (+33%), bicarbonate output (+57%), and lipase secretion (+90%). On the other hand, prolonged (three–four month) estrogen administration triggers an inverse phenomenon. In the second study, carried out in male rats (3), six injections of estradiol benzoate, 50 μg, within a period of 15 days, resulted in a significant increase of all basal bile-pancreatic juice parameters, an increase most marked in lipase secretion (+118%). These studies implied that estrogens might participate, either directly or indirectly, in the "pancreon" secretin response and, thus, would imply postsecretin differences in exocrine pancreatic secretion, particularly in aged women, in whom there is a significant decline in estrogen levels (4, 5). This study, therefore, compares the secretin-induced pancreatic response of normal men and women over and under the age of 45 years.

Materials and Methods

Secretin tests (Boots, 1.0 U/kg intravenous bolus) were performed in 38 women and 46 men, stratified in two groups by age—over 45 years and under 45 years. In the former group, there

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TABLE 1

Hydrelatic and Ecbolic Secretory Response to Secretin in Men

Average values, 80 min	Men under 45 years* (N = 26)			Men over 45 years† (N = 20)				
	Average	SEM	Coef. Var. %	Average	SEM	Coef. Var. %	*t*	*p*
Volume								
Total (ml)	232	14.53	32.0	244	21.96	40.0	0.60	NS
Total (ml/kg)	3.14	0.19	30.0	3.38	0.32	43.0	0.67	NS
Bicarbonate								
Peak (mEq/l)	114	1.72	8.0	115	3.18	12.0	0.17	NS
Total output	19.75	1.25	32.0	21.14	2.04	43.0	0.61	NS
Lipase								
Total output (U/ml)	106000	13749	55.0	214000	44000	71.0	4.17	<0.001
Total output (U/kg)	1389	174	55.0	3003	689	77.0	2.7	<0.01
Amylase								
Total output	1334	115	42.0	1821	186	40.0	2.34	<0.025
Total output (U/kg)	17.90	1.46	40.0	24.21	2.73	44.0	2.19	<0.05

* Average age: 34.0 ± 1.40; range: 21–44.

† Average age: 55.08 ± 1.77; range: 47–81.

were 20 tests in males (mean age 55.05 ± 1.77, range 47–81 years) and 18 tests in females (mean age 51.5 ± 0.98, range 45–62 years). In the series under 45, there were 26 tests in males (mean age 34.7 ± 1.40, range 21–44 years) and 20 in females (mean age 36.65 ± 1.13, range 24–43 years).

The mean body weight in each group was the following: females, under 45 years, 62.15 ± 2.25 kg; over 45 years, 67.90 ± 2.10 kg (difference not

TABLE 2

Hydrelatic and Ecbolic Secretory Response to Secretin in Women

Average values, 80 min	Women under 45 years* (N = 20)			Women over 45 years† (N = 18)				
	Average	SEM	Coef. Var. %	Average	SEM	Coef. Var. %	*t*	*p*
Volume								
Total (ml)	208	15.36	33.0	191	13.24	29.0	0.82	NS
Total (ml/kg)	3.35	0.29	38.0	2.83	0.20	29.0	1.47	NS
Bicarbonate								
Peak (mEq/l)	107	2.0	8.0	98	4.34	18.0	2.07	<0.05
Total output	17.07	1.29	34.0	13.53	1.46	46.0	1.79	NS
Lipase								
Total output (U/ml)	76034	14984	77.0	83673	38962	46.0	0.61	NS
Total output (U/kg)	1398	213	73.0	1215	133	46.0	0.07	NS
Amylase								
Total output	1381	164	52.0	1375	139	42.0	0.03	NS
Total output (U/kg)	22.88	0.99	56.0	20.29	1.95	40.0	0.70	NS

* Average age: 36.65 ± 1.13; range: 24–43.

† Average age: 51.5 ± 0.98; range; 45–62.

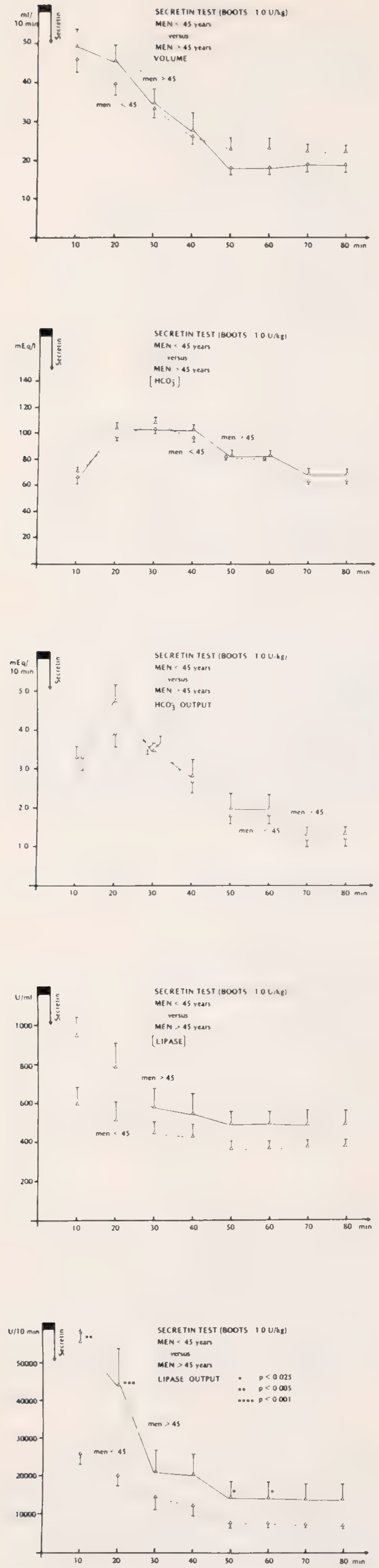


Fig. 1. Postsecretin secretory patterns, men under and over the age of 45 years.

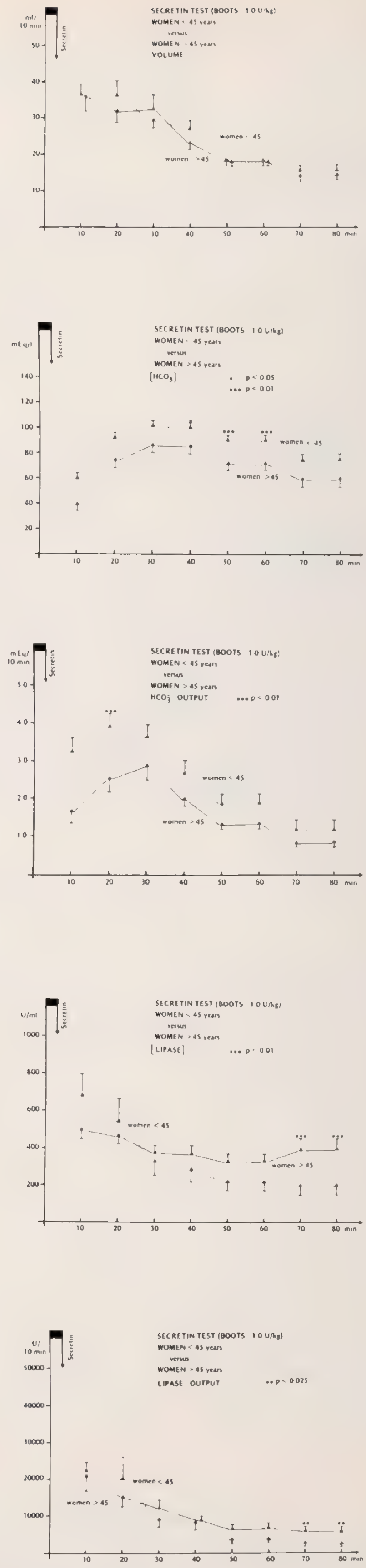


Fig. 2. Postsecretin secretory patterns, women under and over the age of 45 years.

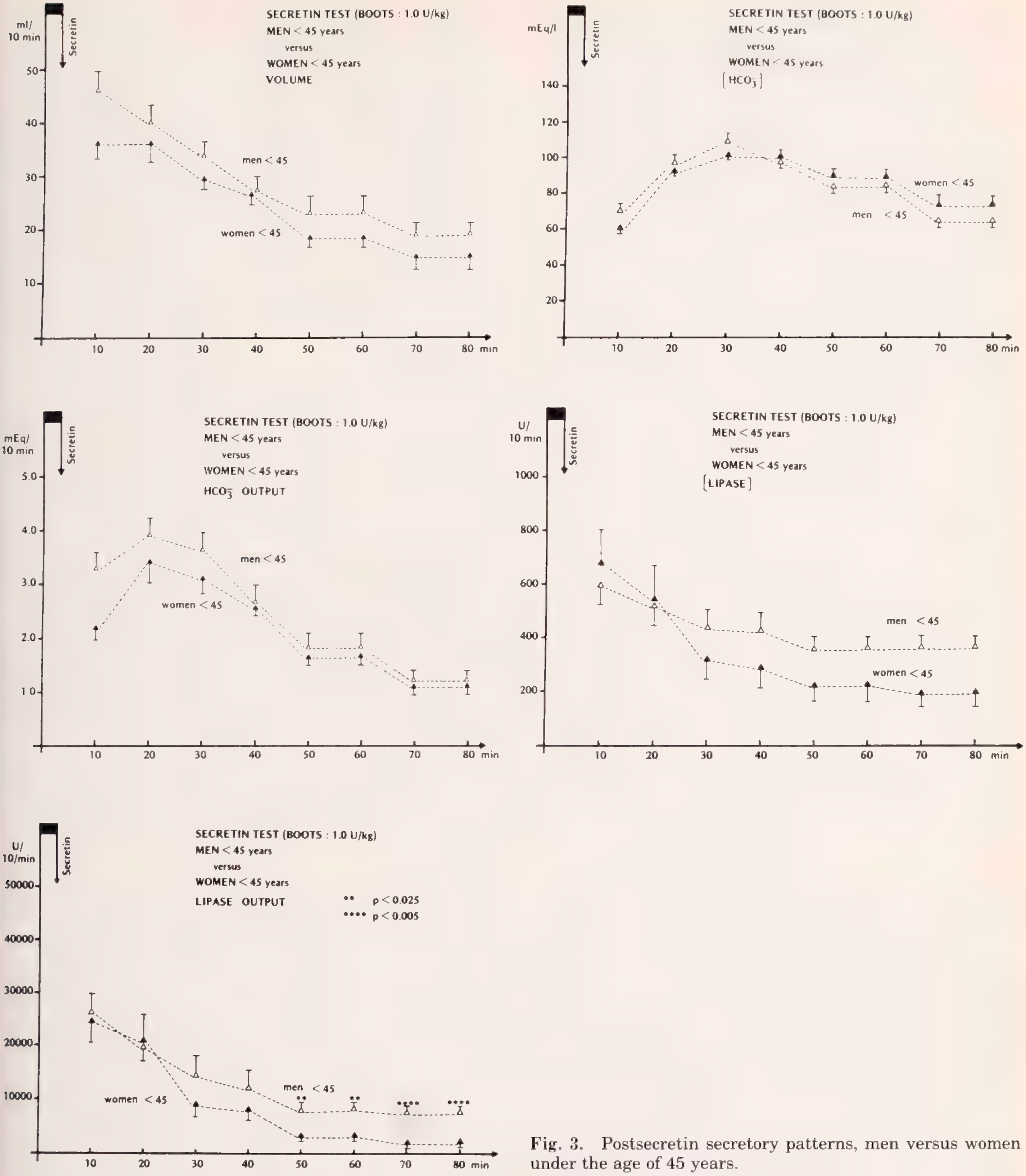


Fig. 3. Postsecretin secretory patterns, men versus women under the age of 45 years.

statistically significant); males under 45 years, 74.04 ± 1.99 kg; over 45 years, 73.85 ± 3.03 kg (difference not statistically significant). When comparison is made between the body weights of men and women, the difference ($p < 0.05$) in the group under 45 but not over 45 years is statistically significant.

The secretin test was performed according to the technique of Dreiling (6–8). After duodenal intubation with a double-lumen tube (Dreiling), gastric and duodenal juices were collected separately. After a 30-minute control collection period, Boots secretin was injected as a bolus at the standard dose of 1.0 U/kg. The duodenal aspirate was collected by intermittent suction over the next 80 minutes in divided 10-min periods. The 80-min cumulative values were also analyzed.

The hydrelatic and ecbolic responses were measured by the following parameters: volume, ml/10 min; pH; bicarbonate, concentration, mEq/1, by the titrimetric method with nitric acid of Lehmann et al (9); lipase concentration, by the method of Lehmann et al (10); amylase concentration in the 80-min secretory pool by the Somogyi method (6–8).

The parameters analyzed statistically were the following: volume, ml/10 min, ml/80 min, ml/kg; bicarbonate concentration, mEq/1 in each 10-min period and peak value in 80 min; bicarbonate output, each 10-min period and the 80-min cumulative values; lipase, U/ml of each 10-min period; lipase output, each 10-min sample and the 80-min cumulative values and units per kg (U/kg). Amylase output, 80-min total and per kg (U/kg).

In both men and women, over and under 45 years, the 80-min average values as well as the maximal and minimal control values were determined (Tables 1, 2). In each of the 4 groups, the peak 15-min bicarbonate output per gram of pancreas was calculated. For this determination, the average pancreatic weight values for both sexes calculated by Schaefer et al (11) were accepted.

Statistical analysis was by Student's *t* test (12) for nonpaired values; $p < 0.05$ was accepted as statistically significant.

Results

Men under versus Men over 45 Years. Analysis of the values of all parameters in each of the eight 10-min periods postsecretin (Fig. 1) revealed no significant differences in pancreatic flow, bicarbonate concentration and output of the duodenal juice collected after submaximal intravenous secretion stimulation (Fig. 1). Lipase concentration and outputs during the postsecretin collections are consistently higher in the men aged over 45 years than in the younger men. The differences are statistically significant only for lipase output during the first two 10-min postsecretin collections (Fig. 1).

In the 80-min cumulative data (Table 1) post-secretin pancreatic flow, bicarbonate concentration, and output are consistently higher for men over 45 years of age than for men under 45 years. The differences are not statistically significant. On the other hand, the 80-min cumulative outputs of both lipase and amylase are statistically significantly greater in the group of men aged over 45 years.

Women under versus Women over 45 Years. In the data on all parameters in each of the eight 10-min postsecretin periods (Fig. 2), pancreatic postsecretin flows are similar in women above and below 45 years. Bicarbonate concentrations and bicarbonate outputs are consistently lower in women over 45 than in women under 45. Some of the differences are statistically significant. Lipase concentration and lipase output in women over 45 years both tend to be elevated above those parameters in women under 45. For both concentration and output, the differences are statistically significant only in the last two collection periods.

In the cumulative data for all parameters comparing women above and below 45 years of age (Table 2), the 80-min rate of flow, fluid output, bicarbonate output, and lipase and amylase outputs are similar for both age groups. Peak bicarbonate concentration was statistically significantly higher in the age group over 45.

Men under versus Women under 45 Years. The postsecretin collection data comparing men and women under 45 years (Fig. 3) shows that pancreatic flow, bicarbonate concentration, and bicarbonate output are similar for both sexes. Lipase concentrations and outputs are higher in men of this age group than in women. Only the data for output is statistically significant.

In the 80-min cumulative data for men and women under 45 years (Fig. 4), none of the parameters show differences that are significant. However, decreased lipase output in women is borderline.

Men over versus Women over 45 Years. The postsecretin collection data comparing men and women over 45 years of age (Fig. 5) show that values for women are consistently less than values for men in this age group. Some parameters, particularly lipase concentration and output, but not all, are statistically significantly different.

In the cumulative 80-min data for men and women over 45 (Fig. 6), all parameters are consistently less for women than for men. The data, however, show a statistically significant difference only for bicarbonate concentration, bicarbonate output, lipase concentration, and lipase output.

Discussion

The present findings confirm previous observations that human exocrine pancreatic response to hormonal stimulation shows variation linked to sex and age. These alterations in pancreatic secretory parameters (Table 3) are most promi-

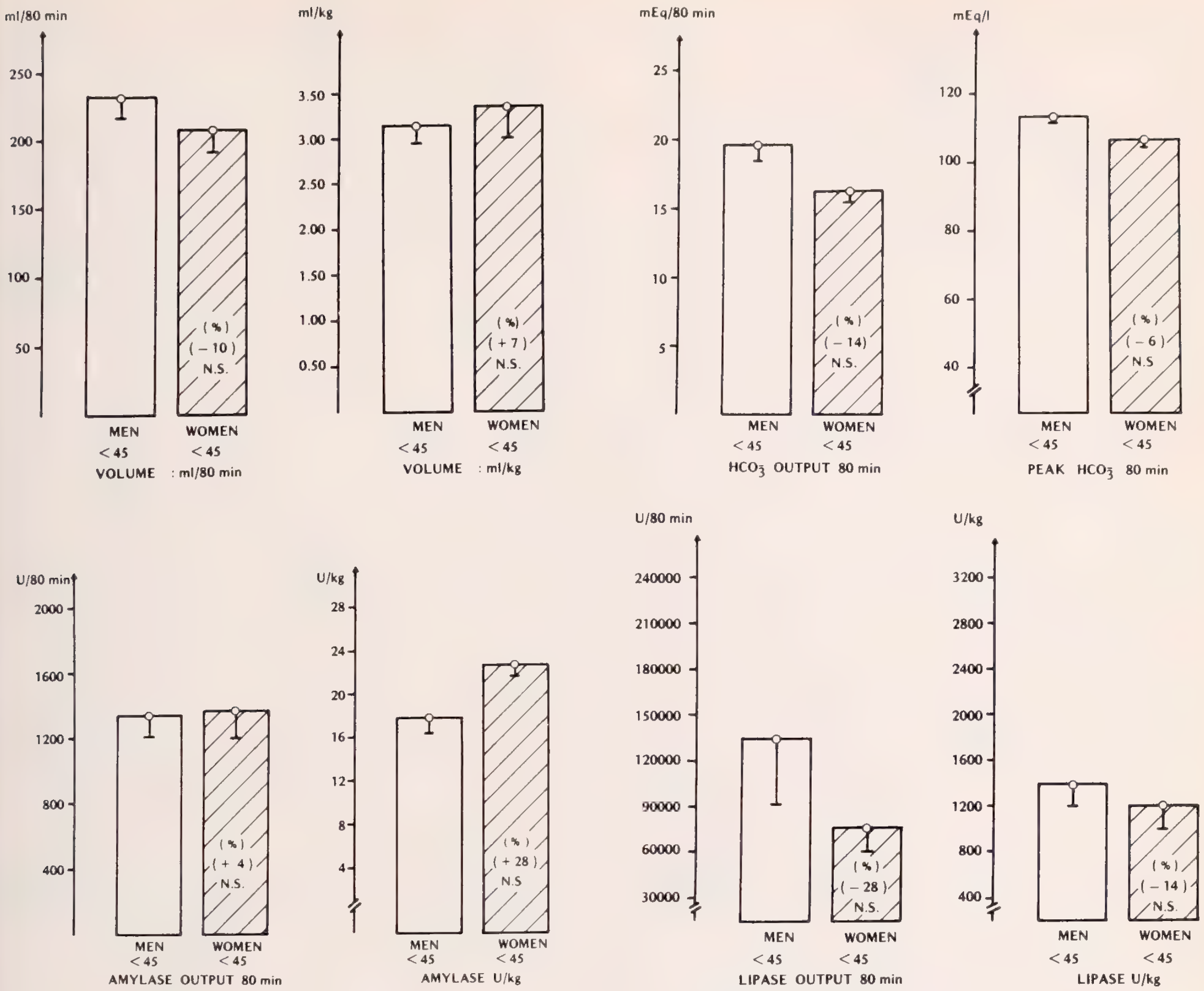
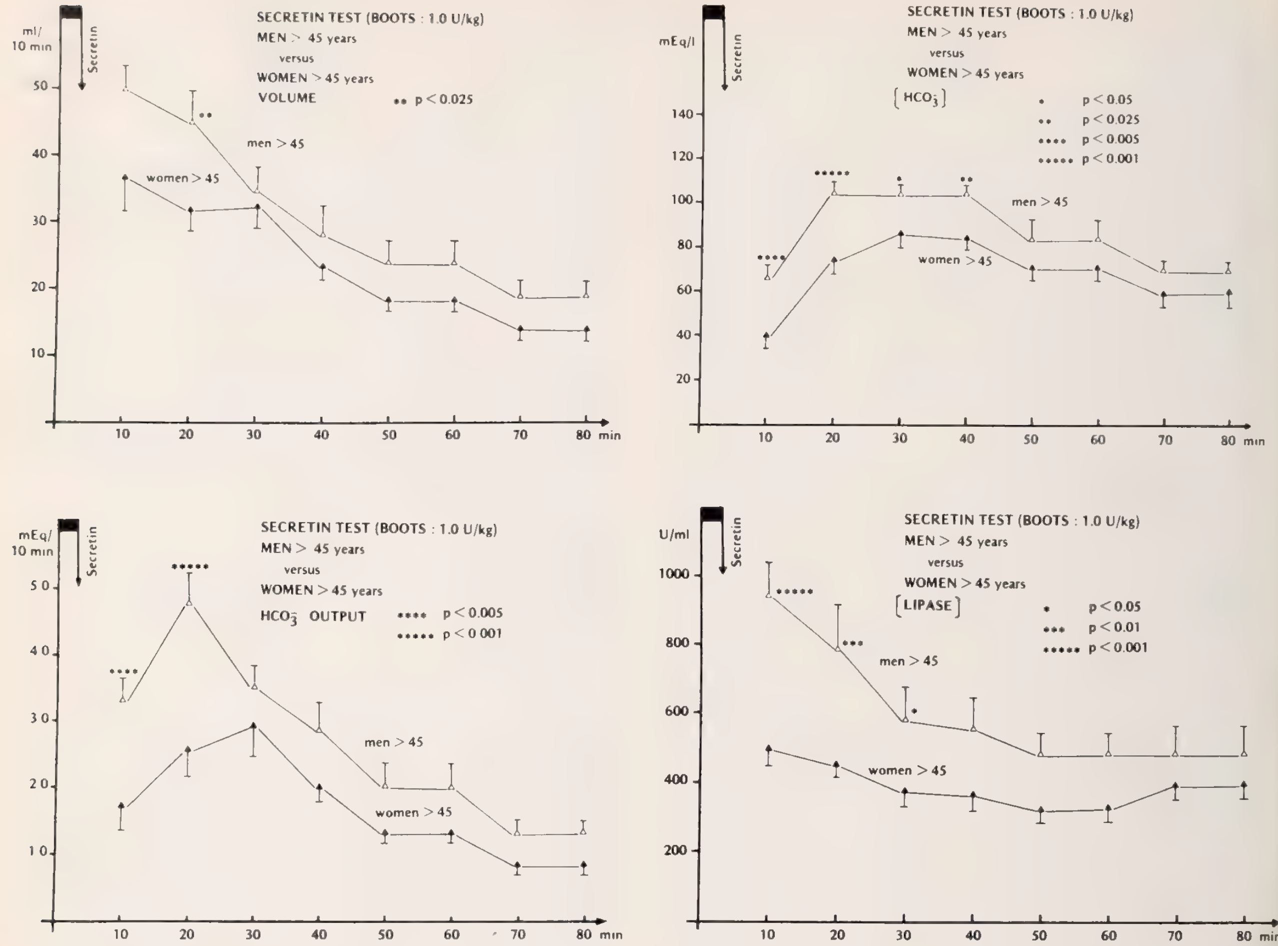


Fig. 4. Postsecretin 80-min cumulative values, men versus women under the age of 45 years.

nent in the group over 45 years of age as well as in women compared with men. In general, the secretion of fluid is not significantly changed in men after 45 years, tends to be greater in men than in women, and is significantly diminished in women after 45.

The ecbolic function of the pancreas shows age, sex, and enzyme variation. Lipase secretion appears affected by age and sex; amylase is not. In both men and women, lipase concentration and secretion are significantly increased in the group over 45 years. Lipase secretion calculated per milligram pancreas is statistically less in women than in men.

The present results—that men over 45 years of age have a greater postsecretin exocrine pancreatic secretory capacity than women—are in agreement with previous observations of Christensen (13) and Perrier (14). Both investigators, working with Vitrum secretin at the submaximal dose of 1.0 U/kg, found significantly higher hydrelatic values in men than in women. Hansky et al (15), in Dreiling's laboratory, attempted to compare their results in the dog with some in humans. To this end, in 12 randomly selected normal subjects, six males and six females, the response to a standard secretin test (1.0 U/kg) was analyzed. The mean peak 15-min bicarbonate output was 6.9 mEq (range, 5.5–8.1) in the women and 8.2 mEq (range, 6.1–12.9) in the men. The calculated bicarbonate output per gram of human pancreas was 0.083 mEq/gm in the females and 0.09 mEq/gm in the males, which was consistent with the observation that a female dog excreted the same amount of bicarbonate (0.08 mEq/gm) as the female human. A similar analysis discloses that the bicarbonate output per gram of gland is greater in men than in women and that the difference gets larger in the subjects over 45 years. Indeed, under 45 years, the bicarbonate output per gram of gland is 0.064 mEq in men and 0.058 mEq in women (percentage differ-



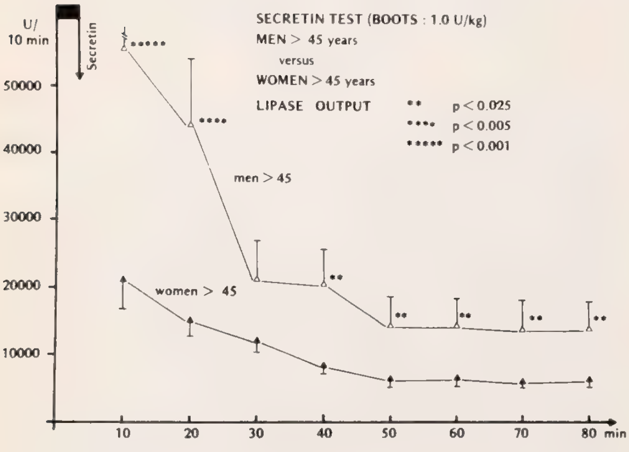


Fig. 5. Postsecretin secretory patterns, men versus women over the age of 45 years.

ence, 9.4); over 45 years, the respective values are 0.073 mEq and 0.047 mEq (percentage difference, 36). Further scrutiny of the data indicates that, in men, the bicarbonate output per gram of gland increases with age and that, in women, the inverse phenomenon develops. Finally, not only is exocrine pancreatic secretion in response to exogenous stimulation less in women than in men, but Baron (16) has reported a similar phenomenon for gastric secretion. With the histamine test, he found an average acid output of 21.6 mEq/h in men compared to 12.3 mEq/h in women.

In order to clarify the physiopathogenic mechanisms underlying the observed secretory differences between men and women, we note the neuroendocrine changes that develop perimenopause and postmenopause (4, 5) in women as well as in the equivalent stage of andropause in men.

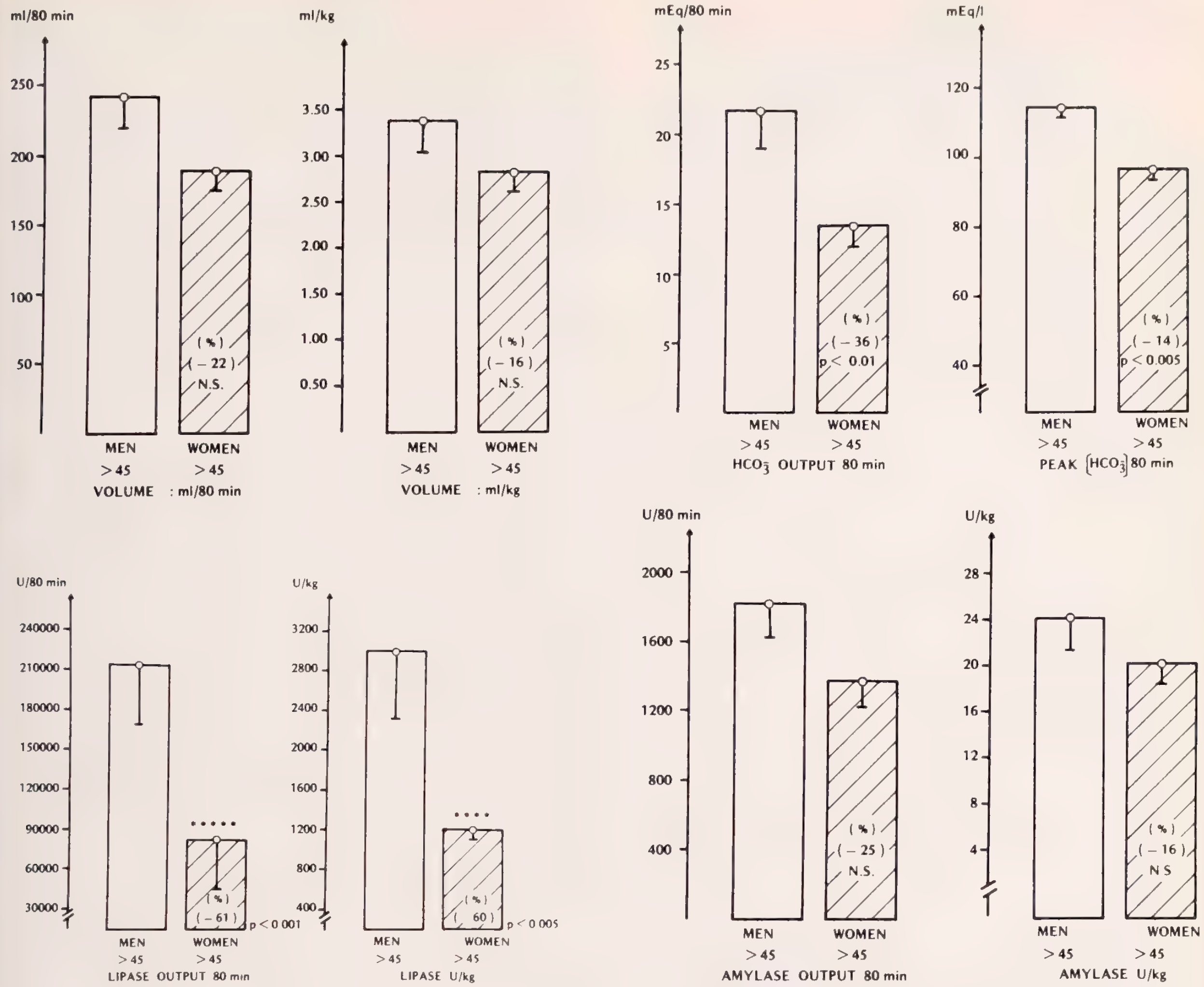


Fig. 6. Postsecretin 80-min cumulative values, men versus women over the age of 45 years.

In women, follicular depletion of the ovaries and exhaustion of their gametogenic and endocrine functions are somewhat precocious events that result in irregular, frequently anovulatory, cycles and, finally, in menopausal amenorrhea. Within two years after the menopause the ovaries secrete only insignificant quantities of estrogens but continue to secrete substantial amounts of androgens. Estrone becomes the major circulating postmenopausal estrogen; it arises almost entirely from peripheral conversion of androstenedione. The latter hormone originates mainly from the adrenals and, to a lesser extent, from the ovaries. The decrease in circulating estrogens after menopause is attended by a dramatic elevation in blood levels of FSH and LH, reflecting an increased production of these gonadotropins in the absence of a negative feedback system. Following bilateral oophorectomy, plasma estradiol falls rapidly to one-tenth, plasma estrone to one-half, and total urinary estrogens to one-fifteenth of preoperative levels, and all continue at low plasma concentrations. Concurrently, plasma androstenedione levels fall to one-half of antecedent concentrations, but plasma testosterone remains unchanged. Thus, it is evident that oophorectomized women are estrogen deficient. FSH levels rise progressively in the first six months after oophorectomy, reaching a maximum of four times or more the preoperative level, and LH similarly rises, to double or more. This comparatively greater increase in FSH over LH results in FSH/LH ratios of >1. This endocrine profile after oophorectomy is rather similar to the hormonal status of postmenopausal women. However, castration after the menopause results in an additional increase in gonadotrophin concentrations. A protein hormone called inhibin, which originates in the gonads and regulates FSH secretion, has recently been described. Inhibin is synthesized by Sertoli cells in the male and by granulosa cells in the

TABLE 3
Postsecretin Secretory Changes (Secretory Pattern and 80-min Cumulative Values by Sex and Age)

	Men <45 years	Men >45 years	Women <45 years	Women >45 years
Volume	→	→	→	→
Bicarbonate	→	→	→	↓
Bicarbonate output	→	→	→	↓
Lipase	→	↑	→	↑ ↑
Lipase output	→	↑ ↑	→	↑
Amylase output	→	↑	→	→
	Men <45 years	Women <45 years	Men >45 years	Women >45 years
Volume	→	→	→	↓
Bicarbonate	→	→	→	↓ ↓
Bicarbonate output	→	→	→	↓ ↓
Lipase	→	→	→	↓ ↓
Lipase output	→	↓	→	↓ ↓
Amylase output	→	→	→	→

→ Change not significant
↑ Increase significant
↑ ↑ Increase highly significant
↓ Depression significant
↓ ↓ Depression highly significant

female. Injected immediately after castration in the rat, inhibin prevents the FSH rise by a direct action at the pituitary level.

Concerning the neuroendocrine changes in men (5), whereas testosterone is almost exclusively secreted by the testis, other androgens and some estrogens come from the adrenals and/or from peripheral conversion of precursors. Spermatic venous blood levels of testosterone, androstenedione, dehydroepiandrosterone, androstenediol, and dehydrotestosterone show a significant negative correlation with age. On the other hand, the increase of estradiol plasma concentrations found by several authors in aging males seems to be associated with decreased metabolic clearance and an increase in peripheral aromatization of androgens.

How can these changes in hormonal profile be correlated with the pancreatic secretory differences between men and women, especially after the age of 45? Two possibilities must be considered. Either the basis for the relationship lies in the pancreas itself and/or in the digestive tract, or the differences result from modifications of the hypothalamic-pituitary-gonadal axis which trigger alteration of the normal interplay between the estrogenic tone and the secretin receptors on the pancreon cells. According to the first hypothesis, pancreatic polypeptide might be involved. This peptide, mainly an inhibitor of pancreatic secretion, increases in plasma concentration as age advances (17). Whether or not this increase is sex-linked has not been elucidated. An alternative possibility is that in women, the activation of the pancreon trigger zone, the duodenum, is weaker than in men due to a much lower gastric acid secretory capacity in women (16). Indeed, with increasing age, there is a progressive decline in gastric acid secretion. Again, it is as yet unknown if the degree of depression that occurs with age is different in the sexes. A final consideration is the formation within the intestinal lumen of the potentially antiestrogen agents. Indeed, recent work has shown (18) that a group of compounds called lignans—enterolactone and enterodiol—are formed in the intestinal lumen by bacteria from precursors present in fiber-rich food. Enterolactone has a structure resembling synthetic estrogens such as stilbestrol and hexestrol, but show no estrogenic activity. On the contrary, these compounds have antiestrogenic properties, activities which can alter the sensitivity of the secretin receptors of the pancreon cells. Such an effect, of course, leads to the second hypothesis. Indeed, the pancreon cells have estrogen receptors in much higher numbers than in other organs (19). It is not known whether these cytosolic receptors are present in both the acinar and centroacinar-ductal cells. Recent studies have shown that there are also estrogen receptors in the smooth muscle of the pancreatic vessels and in the majority of the cells of the islets of Langerhans (20). On the other hand, androgen receptors were found only in the interstitial connective tissue of the pancreatic gland (20). It has been speculated that estrogenic tone, that is, activation of the estrogen receptors, could modulate the response of the pancreon receptors to secretin, CCK-PZ, acetylocholine, and so on. Thus, decreased estrogen activity in the menopause could account for the secretory decline after secretin stimulation in women over 45. Furthermore, it follows that in men over 45 years, a relative intrapancreatic hyperestrogenic tone could explain the higher hydrelatic secretion and the significantly enhanced ecbolic response following secretin stimulation. The intrapancreatic hyperestrogenic tone in men over 45 years would be the result of a progressive fall of testosterone levels, coupled with a constant secretion of estradiol (4, 5).

Acknowledgments

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Estrogen Effects on Basal Bile-Pancreatic Secretion and the Exocrine-Endocrine Pancreatic Gland in the Rat

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Abstract

To gain insight into certain clinical events—functional disturbances of the digestive tract during the menstrual cycle, pregnancy, and the puerperium, and after administration of contraceptive agents, and the development of chronic pancreatitis during the menopause—we determined the effect of parenteral estrogen administration on rat bile-pancreatic section. In male rats, subcutaneous administration of estradiol benzoate (50 μg) every other day for two weeks induced an increase of the hydrelatic and ecbolic parameters of basal bile-pancreatic secretion. These changes became evident on the second day of estradiol administration. Cumulative 180-min secretion collection disclosed significant augmentation of flow (+20.7%), protein output (+329%), and lipase output (+111%). Simultaneous plasma determinations displayed a significant increase of prolactin (+900%), glucose (+53%), triglycerides (+13.4%), cholesterol (+29.0%) and total lipids (+4.2%). Histologic examination of the exocrine and endocrine parenchyma disclosed structural change. In the exocrine parenchyma there was a marked depletion of zymogen granules in the acinar cells. The Langerhans islets were reduced in size and in cell mass following an initial period of hypertrophy.

Estradiol-evoked bile-pancreatic secretion potentiation may be the result of either a direct or an indirect mechanism. A direct mechanism would be a consequence of activation of the cytosolic estrogen receptors in hepatocytes and "pancreon" cells. Such an action would elicit potentiation of normal interaction with receptors responsive to the neuroendocrine hormones that normally induce basal bile-pancreatic secretion. An indirect mechanism could result from estradiol-evoked release of insulin which could intensify the secretory effects of CCK-PZ and acetylcholine on the "pancreon" units.

Functional disturbances of the digestive tract occur during the menstrual cycle, pregnancy, and the puerperium, and following the administration of contraceptive drugs (1–4). Moreover, chronic pancreatitis without obvious etiology has been observed to develop during menopause. This study was undertaken to gain insight into mechanisms possibly causing these clinical events by determining the effect of parenteral estrogen administration on rat bile-pancreatic secretion.

Materials and Methods

The experiments were performed in male Wistar rats weighing between 250 and 400 gm (Table 1). The experimental group of 13 animals was subjected to subcutaneous injection of 50 μg of estradiol benzoate in 0.1 ml peanut oil solvent.

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Estrogen was administered every other day for 15 days. In a control group of 13 animals, only the subcutaneous estrogen solvent was given for the same period. The average weight of both groups was the same.

Biliary Pancreatic Fistula. A biliary pancreatic fistula was created by modifying the technique described by Cavarzan et al (5) (Fig. 1). After entering the abdomen through a midline incision, and under the dissecting microscope, a plastic catheter (outside diameter 0.65) was inserted in the terminal end of the bile duct. The distal segment of the duct was ligated with 6–0 silk and the catheter secured with another ligature of the same material.

One of the main modifications of Cavarzan's technique consisted of giving the draining catheter a hook form. The tip of the short segment of the hook was bevelled to facilitate its entrance into the bile duct. Close to this tip, a small hole was made in the catheter opposite the bevelling. This was done to prevent interruption of the bile-pancreatic flow if the bevelled tip were to shift and become blocked by the hepatic duct wall. The long segment of the hook was placed in the subcutaneous tissue at the left side of the midline laparotomy. Elongated by a Silastic catheter, the hook was then brought to the neck through a subcutaneous tunnel.

Another Silastic catheter, 8 cm long, with several holes placed alternately around its circumference, was inserted through a small incision in the posterior wall of the duodenum close to the pylorus. At this level, it was secured by a purse-string suture. It was elongated by another plastic catheter (outside diameter 0.65), then moved on a right-to-left course, and brought to the subcutaneous level through the incision previously made at the left side of the midline laparotomy. From here, it was moved through the subcutaneous tunnel to the neck. The elasticity of the Silastic tube in the duodenum prevented deformation of the duodenal curve. The two catheters in the neck were connected to each other to allow recirculation of bile-pancreatic juice into the gut.

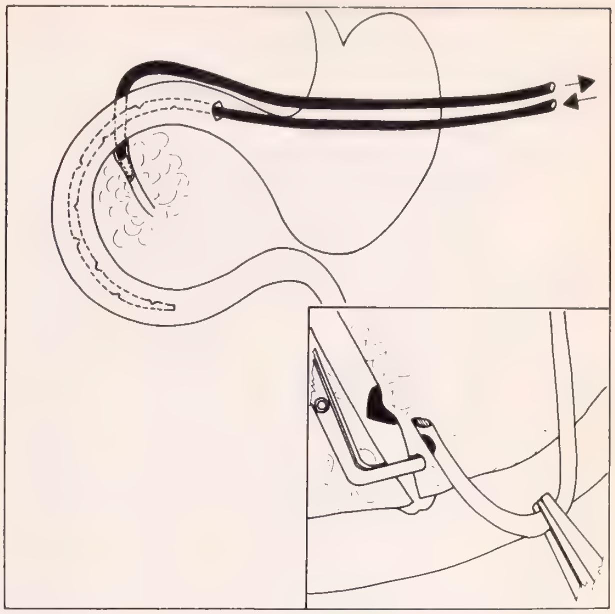

Fig. 1. Schematic drawing of biliary-pancreatic fistula.

This approach has two fundamental advantages. First, it permits unfolding of the mesoduodenum pancreas through which the bile duct courses. This permanent stretching of the mesoduodenum by the intraduodenal Silastic tubing prevents its shinkage and retraction and, thus,

TABLE 1

Weight of Abdominal Organs and Both Testes in Control and Estradiol-Treated Male Rats

	Control		Estradiol-treated		
Weight	*N*	Average ± SE	*N*	Average ± SE	*p*
Body (gr)	13	325 ± 11.67	13	317 ± 10.3	NS
Pancreas					
Head (mg)	13	412 ± 22.85	13	427 ± 24.57	NS
Body-tail (mg)	13	549 ± 38.46	13	586 ± 43.06	NS
Total (mg)	13	961 ± 48.36	13	1013 ± 45.90	NS
Stomach (mg)	13	1314 ± 65.03	12	1374 ± 30.85	NS
Duodenum (mg)	13	920 ± 48.68	12	918 ± 44.81	NS
Small bowel (mg)	13	5491 ± 302	12	5146 ± 244	NS
Colon (mg)	13	2373 ± 156	12	2055 ± 81.37	NS
Both testes (mg)	13	2711 ± 173	12	1903 ± 168	<0.01

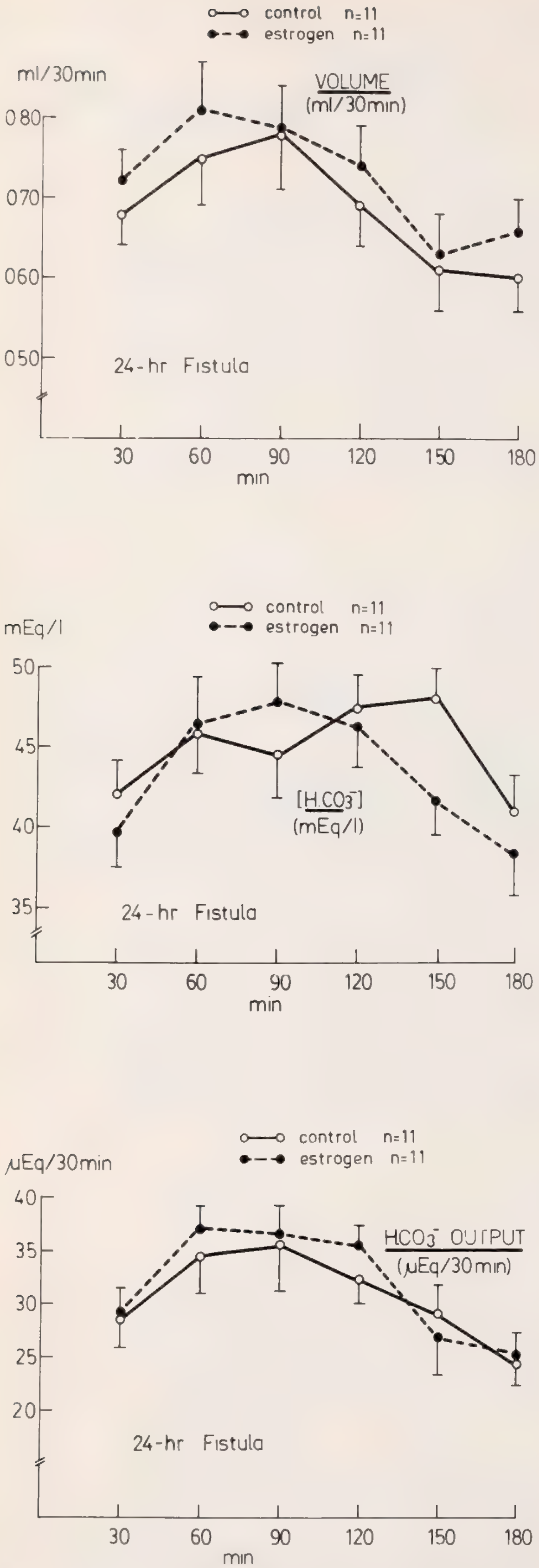

Fig. 2. Rate of flow (ml/30 min), HCO_3^- concentration (mEq/L), and HCO_3^- output (µ/Eq/30 min) of basal bile-pancreatic secretion in control and estrogen-treated rats, 24 hours after fistula was created.

avoids impairment of bile flow and pancreatic drainage through the several small ducts that enter into the distal segment of the hepatic duct. Second, both the tube inserted through the posterior wall of the first part of the duodenum and the hook of the catheter draining the bile duct allow normal positioning of the duodenum, a prerequisite to maintaining unimpaired flow of bile-pancreatic juice to the neck and its recirculation into the upper small bowel.

The surgical procedure was always performed during the morning hours; food was not withheld from the animals beforehand. Between tests, the animals were kept in individual cages.

Rats were tested during the first two days following surgery. Experience has shown that during this period the flow of bile-pancreatic juice is regular and predictable. During the first 48 hours, the animals were allowed to drink tap-water but food was withheld. After 48 hours, when the day's tests had been completed, the rats were allowed to eat. The animals were killed and autopsied 48 to 72 hours after surgery. All abdominal organs were weighed, including the testes.

Basal Bile-Pancreatic Secretion Evaluation. In both animal series, estradiol-treated and solvent-injected, all tests were performed during the morning hours, between 8:00 A.M. and 11:00 A.M., at room temperature (between 25° and 27°C). The rats were placed in a Bollman cage (6). The tubes in the neck were disconnected and the bile-pancreatic juice was collected in small calibrated tubes immersed in crushed ice. The collection periods were 30 min each, and the whole test lasted 180 minutes.

The following parameters and methods were chosen for the biochemical assays: (a) volume (ml per 30 min), measured with a precision of 0.1 ml; (b) bicarbonate concentration, mEq/1, determined by the titrimetric method described by Lehmann et al (7); (c) bicarbonate output, calculated and expressed as µEq/30 min; (d) protein concentration, measured by the method described by Gornall et al (8); (e) protein output, expressed as mg/30 min (f) lipase concentration, determined by the method described by Bang (9), modified by Lehmann et al (10) by converting the original technique to a titrimetic procedure; and (g) lipase output, expressed as Bang-Lehmann U/30 min.

Microscopic Study. During the autopsy of the animals, the pancreatic gland was carefully dissected from the surrounding tissues and the head of the organ was isolated from the body and tail. The head is defined as the segment of the gland

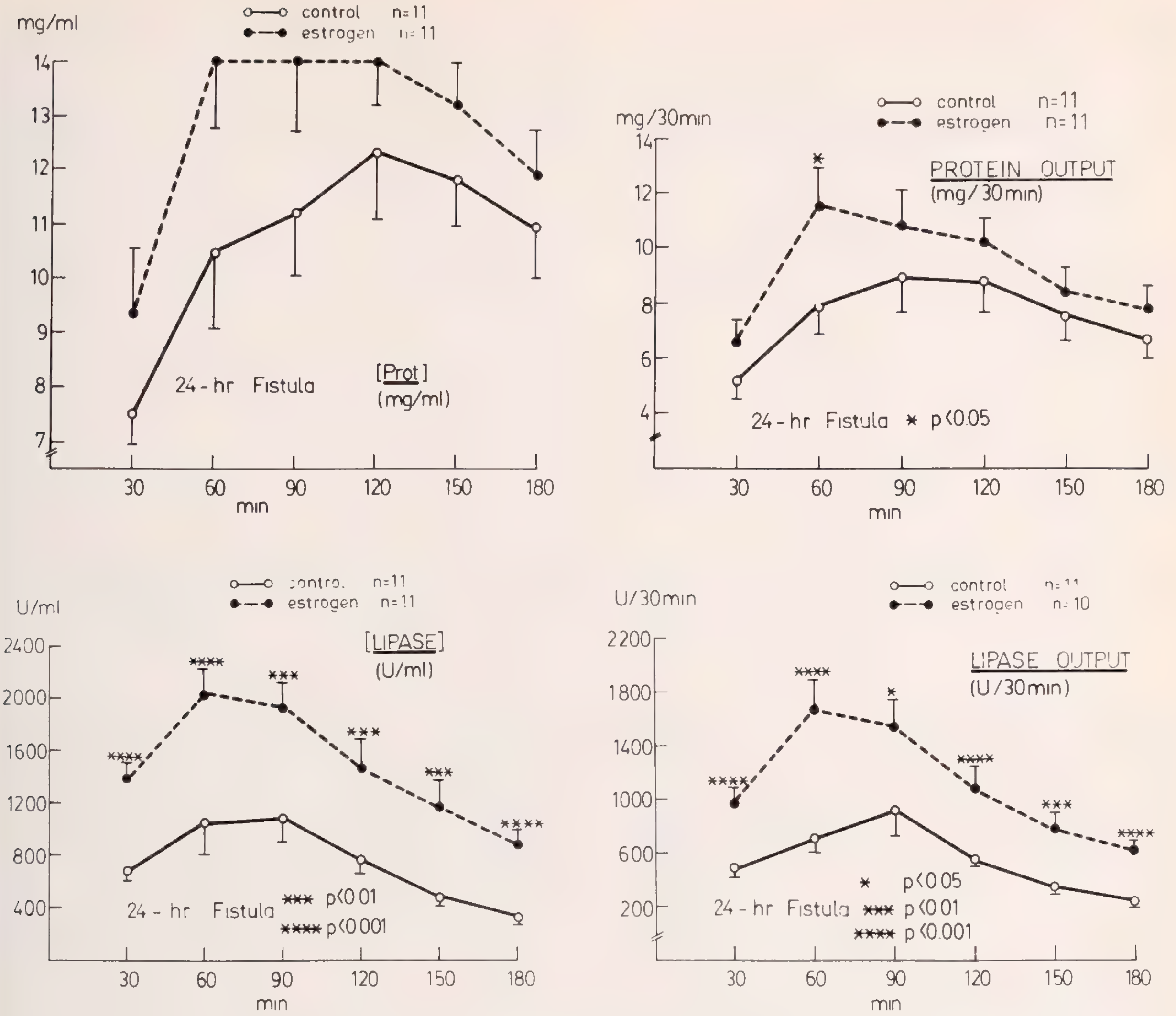

Fig. 3. Protein concentration (mg/ml), protein output (mg/30 min), lipase concentration (U/ml), and lipase output (U/30 min) of basal bile-pancreatic secretion in control and estrogen-treated rats, 24 hours after fistula was created.

between the pylorus, the internal border of the duodenum, and a line drawn along the right border of the mesenteric vein and passing through the pylorus. The remaining gland was considered body-tail. The pancreatic tissue was blotted dry and weighed. The pancreatic gland as well as the rest of the abdominal organs and testes were fixed in neutral formol-calcium. The following staining techniques were used: hematoxylin-eosin, Gomori, and Masson trichrome.

Blood Measurements. Under ether anesthesia, blood was drawn from the heart by transdiaphragmatic puncture. After centrifugation, plasma was separated and kept at −2°C until its processing. Plasma prolactin was measured according to the procedure suggested by the National Institute of Arthritis and Metabolic Diseases, which has provided all the agents for its determination (11). The results are expressed as μg/ml of plasma. The standard reference used was prolactin RP 1. Variations in assays were not greater than 15%. All samples were processed in one single session. Other substances measured were triglycerides, cholesterol, total lipids, and plasma glucose.

Statistical Analysis. Statistical analysis consisted of calculating the mean and standard error of the mean of the results. The significance of the differences between means was evaluated using the Student t test for unpaired values (12). Values of *p* of less than 5% were interpreted as significant.

Results

The experimental series was compared to the control group. Differences were noted in the test results from the two groups at 24 hr and 48 hr after fistula formation. The secretory pattern was

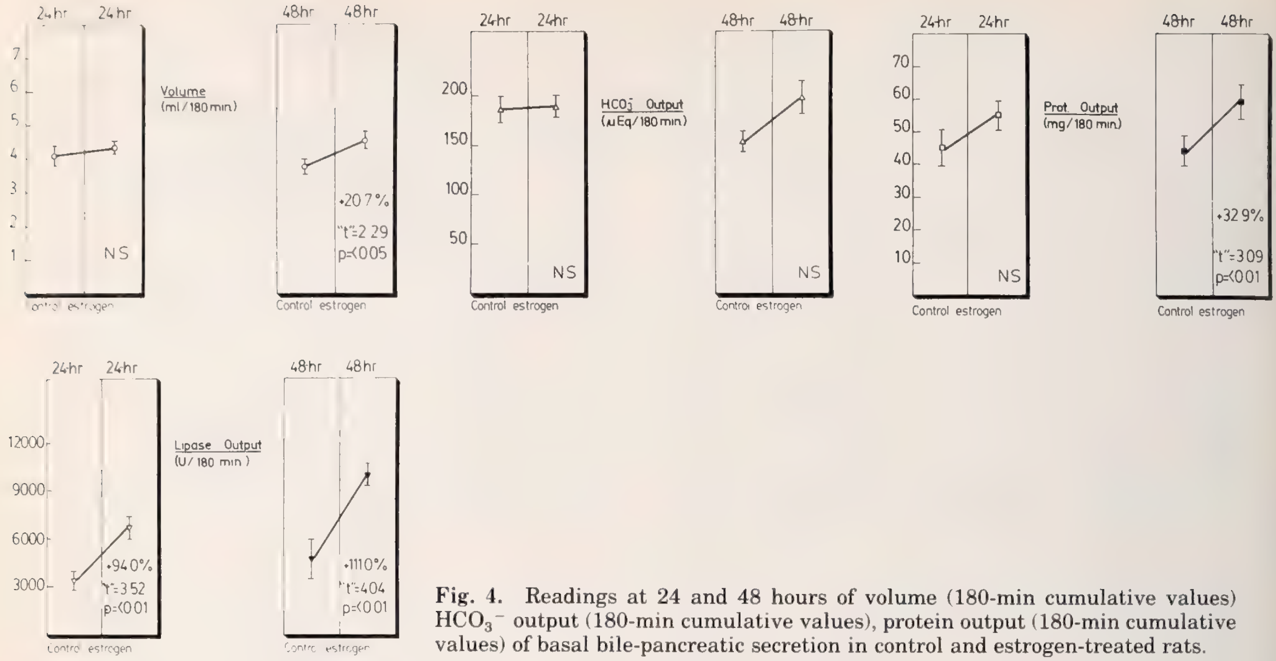

Fig. 4. Readings at 24 and 48 hours of volume (180-min cumulative values) HCO_3^- output (180-min cumulative values), protein output (180-min cumulative values) of basal bile-pancreatic secretion in control and estrogen-treated rats.

studied by comparing data of sequential periods. Secretory rates were calculated and compared as 180-min cumulative values.

Bile-Pancreatic Secretion: Fistula at 24 Hours. After 24 hours, the basal electrolyte pancreatic secretion, that is, the rate of flow and the bicarbonate concentration and output, did not show significant changes (Fig. 2). On the other hand, the estrogen-treated animals' basal protein concentration and secretion were greater than in control rodents. The protein output data were statistically significant, especially for lipase (Fig. 3).

The 180-minute cumulative data comparing estrogen-treated and control rodents disclosed no differences in the pattern of secretion (rate of flow and bicarbonate output). Basal ecbolic secretion was augmented for protein and lipase output, but only the latter was statistically significant (Fig. 4).

Bile-Pancreatic Secretion: Fistula at 48 Hours. At 48 hr, the estrogen-evoked basal secretory changes were more marked. Both the hydrelatic and ecbolic bile-pancreatic secretion values, in comparison to those of the control series, disclosed either a tendency to increase, as in the bicarbonate and protein concentration, or significant increases, as in flow, bicarbonate output, and lipase concentration and output (Figs. 5, 6).

The 180-min cumulative basal secretory patterns, that is, flow, and bicarbonate concentration and output, as well as the ecbolic responses of protein and lipase secretion were more marked than at 24 hr, comparing estrogen-treated and solvent-treated rodents. The increases in all parameters at 48 hr in the estrogen-treated group were statistically significant (Fig. 4).

Plasma Responses to Estrogen. Comparison of the plasma levels of prolactin, glucose, triglycerides, cholesterol, and total lipids of estrogen-treated and control rodents showed large and statistically significant increases in these substances in the treated animals (Table 2).

Pathology. Almost all of the estradiol-treated animals showed marked turbidity of bile-pancreatic secretion at 48 hr. This phenomenon was only occasionally seen in control animals. The turbidity change was evident within the first 60–90 min after starting secretory collection. Microscopic study of the sediment generally disclosed an abundance of calcium carbonate, cholesterol, and calcium bilirubinate crystals. Calcium concentrations in the bile-pancreatic secretion were greatly increased following dissolution of the floccules by heat.

Comparison of the weights of the abdominal organs of control and estrogen-treated rodents showed a 30% reduction of testes weight in the treated animals; no other statistically significant differences were observed (Table 1) between the two groups.

Histologic study revealed alterations in the liver, pancreas, and testes of the estrogen-treated rodents. The liver showed slight steatosis of the small vacuoles and a lymphocytic infiltration. Compared to controls, the bile duct wall was thinner. Its surface epithelium was cylindric or cubic. Large perinuclear vacuoles had the peculiar appearance of empty cells. There was no metaplasia. The crypts were short, with a wide-open outlet into the bile-duct lumen. The epithelium of the crypts was tall, with Alcian-blue-positive mucus. In the duct surface epithelium, some papillas were found with clear cells. The "pancreon" cells looked relatively clear and vitreous, without the classical granular appearance. Many of the pancreocytes appeared empty, similar to what is seen after pilocarpine injection. The empty zones were irregular; they comprised a whole lobule or part of it. The Langerhans islets' cells were smaller than those in the control glands, with a reduction in the number of cell ribbons detailed. Morphometric analysis, the subject of another report, disclosed that after six estradiol injections, the islets' diameters were reduced by half. Initial lesions of the testes consisted of necrosis of the primitive spermatic elements in the tubule periphery, resembling a crown of small vacuoles in contact with the tubular membrane. These vacuoles tended to get larger, fusing one to another, and developing into larger lakes. The empty zones collapsed, inducing tubule deformation. In the later lesions, the necrosis was more widespread and left a multinucleated syncytial mass in the centers of the tubules.

Discussion

In the rat, concurrent bile-pancreatic juice collection is technically more reliable than draining these secretions separately. First, placing a ligature in the proximal hepatic bile duct interrupts neural pathways to the liver and pancreas (13). Then, the flow of bile through its duct acts as a suction pump on the flow of pancreatic juice in the small ducts that enter the choledochus. In addition, because of irregular secretory rates, separate collection would require continued testing for 48 hr in order to define the secretory pattern at 24 and 48 hr. Prolonged testing would be inadvisable because of progressive development of a pancreatic inflammatory lesion, probably reflecting injection of the pancreatic tissue via the drainage tube (13). Finally, note that the hydrelatic and ecbolic bile-pancreatic secretion values in control tests differ at 24 hr and 48 hr postoperatively, a reflection of a predominant adrenergic influence in the early post-

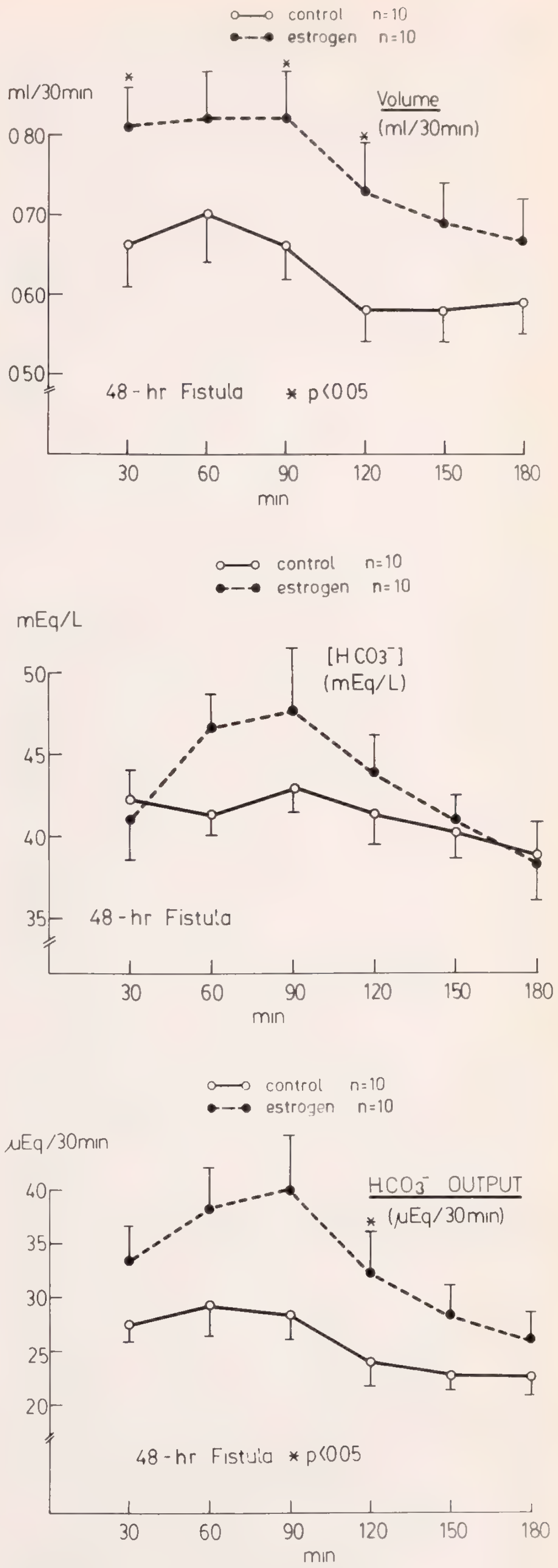

Fig. 5. Rate of flow (ml/30 min), HCO_3^- concentration (mEq/1), and HCO_3^- output (μEq/30 min) of basal bile-pancreatic secretion in control and estrogen-treated rats, 48 hours after fistula was created.

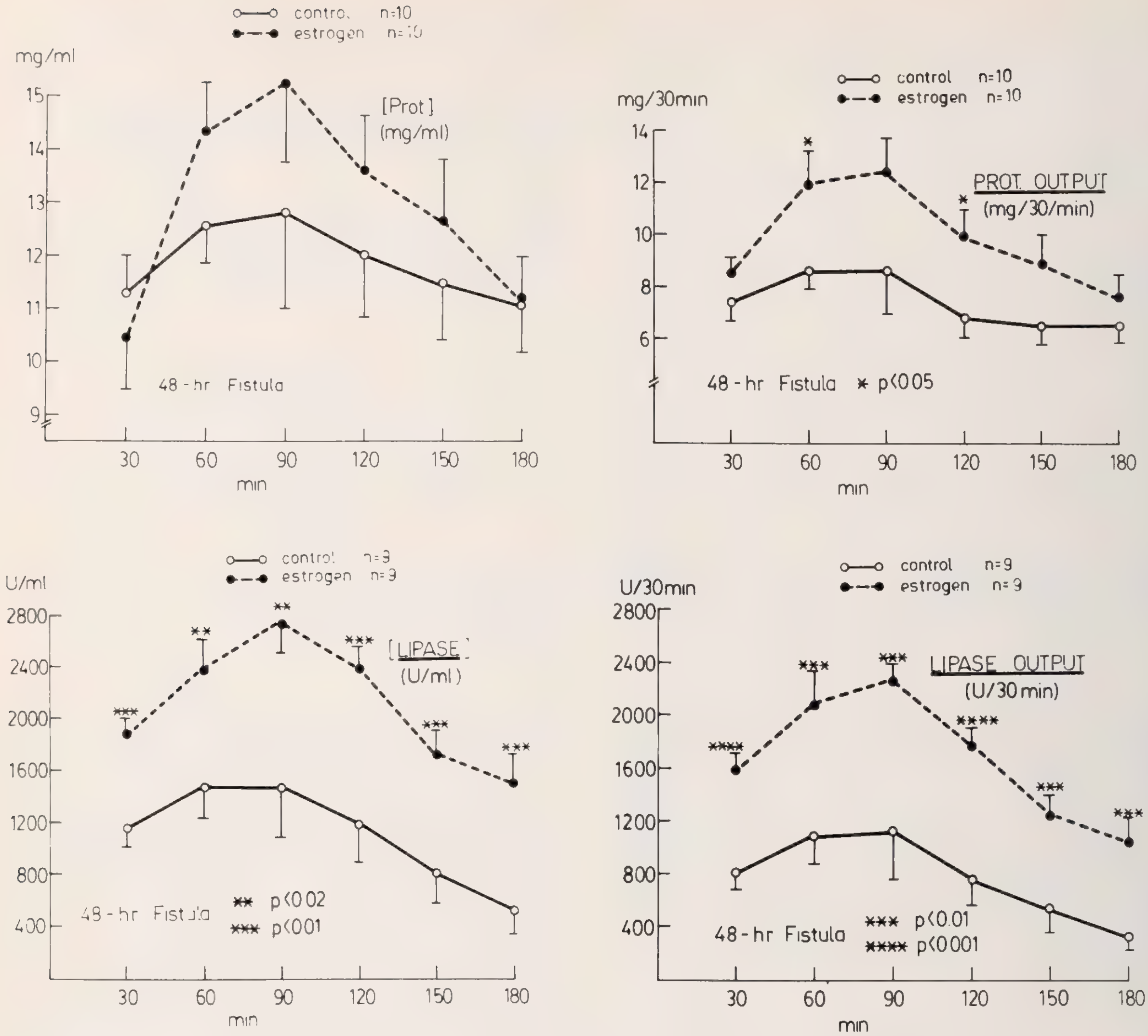

Fig. 6. Protein concentration (mg/ml), protein output (mg/30 min), lipase concentration (U/ml), and lipase output (U/30 min) of basal bile-pancreatic secretion in control and estrogen-treated rats, 48 hours after fistula was created.

surgical hours that, later on, is counterbalanced by a progressive return of cholinergic tone (13).

The dilemma posed by the present data is to explain the function of the estrogen cytosol receptors (14, 15) present in the hepatocytes, the "pancreon" cells, and islet cells, as well as to elucidate the mechanisms by which estradiol treatment induces a potentiation of the basal bile-pancreatic secretion values.

We postulate that normally the intrahepatic and the intrapancreatic estrogenic tone interacts with those neuroendocrine factors that elicit bile-pancreatic secretion, that is, cholecystokinin-pancreozymin (CCK-PZ) and acetylcholine. Thus, estradiol administration would enhance the CCK-PZ and cholinergic effects on the "pancreon" cells (16) and hepatocytes. Moreover, estradiol treatment would, through the activation of the beta cells of the Langerhans islets and the release of insulin, also potentiate CCK-PZ and acetylcholine activity (17). Our observations confirm the reports of others (18, 19) that estradiol administration induces marked hypertrophy of beta cells, accompanied by progressive reduction of islet size and cell mass.

The marked increases in serum prolactin following estrogen administration in the rat are at variance with Rosenberg and Dreiling's data in canine studies (4), which indicated inhibition of fluid and electrolytes. Our findings, however, are in accord with the studies of Dreiling et al (3), which noted potentiation of basal and stimulated pancreatic secretion in pregnant dogs.

The estrogen potentiation of pancreatic and biliary secretion at least partially explains the observed liver disorders, including alcoholic cirrhosis (20). The hyperestrogenism resulting from an increased peripheral conversion of testos-

TABLE 2

Analysis of Plasma Levels of Glucose, Triglycerides, Cholesterol, Prolactin, and Total Lipids in Control and Estradiol-Treated Rats

	N	Average ± SE	Difference	*p*
Glucose (mg %)				
Control	10	156.0 ± 8.49		
Estradiol-treated	10	238.8 ± 5.36	+53%	<0.001
Triglycerides (mg %)				
Control	10	78.5 ± 1.29		
Estradiol-treated	10	89.0 ± 0.89	+13%	<0.001
Cholesterol (mg %)				
Control	10	96.4 ± 2.61		
Estradiol-treated	10	124.4 ± 3.44	+29%	<0.001
Total Lipids (mg %)				
Control	10	445.0 ± 6.37		
Estradiol-treated	10	463.5 ± 5.58	+4%	<0.05
Prolactin (μg/ml)				
Control	12	93.5 ± 14		
Estradiol-treated	9	1025.0 ± 218	+1000%	<0.001

terone and androstenedione to estrone (21), coupled with an augmented duct mass responsive to secretin, would result in hypersecretion. Thus, the present results, that is, the selective rise in protein and lipase output (+222.0%) in the estrogen-treated rats, support the speculation that estrogens do potentiate the action of CCK-PZ and acetylcholine in the genesis of basal pancreatic secretion. Conversely, the decline of the intrapancreatic estrogenic tone that occurs in menopause (22, 23), which would induce a decrease in the hydrelatic component of pancreatic secretion, would trigger chronic inflammation in the pancreas of the aged by favoring precipitation of intraductal protein plugs, as described in alcoholic pancreatitis (24–27).

Acknowledgments

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Resolution of Pancreatic Pseudocysts: When Should We Wait?

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Clinical observations (1–3) and experimental studies (4, 5) have indicated that pancreatic pseudocysts may resolve spontaneously. Several investigators (3, 6), using stage of evolution, the size of the cyst, severity of symptoms, and/or the presence of local or systemic complications, have attempted to define criteria for nonsurgical management of pseudocysts. For example, Ravelo and Aldrete (6) suggested that pseudocysts of 5 cm or more in diameter, those that can be palpated externally, and/or those which grossly distort radiographic anatomy should undergo prompt surgery. Others (7, 8), concerned by an approximate 26% rate of preoperative complications (3) and a mortality rate of perhaps 12% (2), continue to advocate surgical drainage.

Abdominal ultrasonography affords accurate diagnosis of pancreatic pseudocysts, early diagnosis of complications, and allows, as well, the opportunity of studying their natural evolution (9–11).

The present report is on a series of six patients with large pancreatic pseudocysts that were unlikely to resolve spontaneously because of their size.

Five of the six patients entered the hospital with attacks of acute pancreatitis of different etiologies. All developed palpable abdominal masses. Pseudocysts of the pancreas were diagnosed by and followed by abdominal ultrasonography. Each patient had an upper gastrointestinal series and serial serum amylase determinations. All patients were maintained on standard total parenteral nutrition (12, 13) until signs of regression of their pseudocyst.

Case Reports

Case 1. A 57-year-old man was admitted to the hospital with abdominal pain, vomiting, and loss of weight over a period of three weeks. Abdominal examination revealed a 15 × 20 cm epigastric mass. The serum amylase level was 684 Somogyi units. The patient had had a truncal vagotomy and antrectomy for a perforated duodenal ulcer one month earlier. Ultrasonogram confirmed the diagnosis of a pancreatic pseudocyst, 21 cm in diameter. Displacement of the stomach and small bowel was evident in the upper gastrointestinal series. Total parenteral nutrition was instituted to treat the pancreatitis and prepare the patient for surgical treatment.

As the patient's clinical and nutritional status improved over a two-week period, the abdominal mass seemed to become much smaller. New sonographic studies showed a significant reduction (50%) of the diameter of the pseudocyst (Fig. 1). The clinical improvement was coincident with a drop of the serum amylase to 386 units. Parenteral nutrition was also slowly reduced and oral feeding started. When the patient was discharged the cyst measured 7 cm. Follow-up sonograms showed complete disappearance of the cyst by week 13.

Case 2. A 39-year-old man was admitted to the hospital with a blunt abdominal trauma sustained in a car accident. At exploratory laparotomy, hematomas in the pancreas and duodenum were found. These areas were drained, with complete recovery at the 10th day after surgery. Forty days later, the patient was readmitted with 10-cm epigastric mass and a temper-

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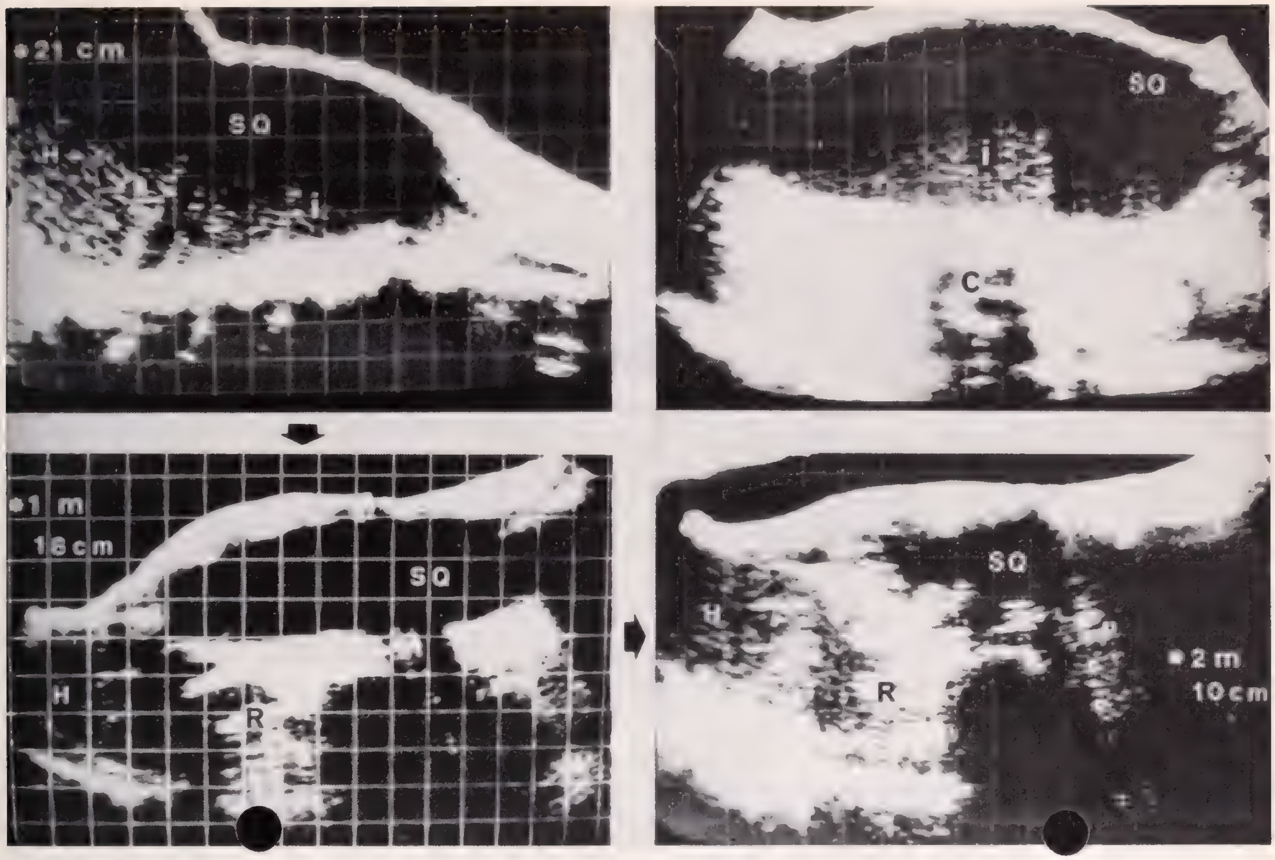

Fig. 1. Case 1. Sonographic demonstration of resolution of pseudocyst from 21 cm to 10 cm.

ature of 38°C. Ultrasound showed a pancreatic pseudocyst of 8 cm in diameter in the tail of the pancreas. The patient was placed on total parenteral nutrition and intravenous ampicillin, administered daily.

One week later, spontaneous reduction of the abdominal mass was noted, coincident with a 50% reduction of cyst diameter in the ultrasonogram (Fig. 2). After the second week of hospitalization, total resolution was complete, ultrasound showing only slight edema of the tail of the pancreas. The serum amylase never went above 400 Somogyi units and regressed to normal. Parenteral nutrition and antibiotics were suspended during the second week of hospitalization.

Case 3. A 26-year-old woman was admitted to the hospital with vomiting and epigastric pain for a week and a palpable epigastric mass of 12 cm in diameter. She had had an attack of acute pancreatitis one year previously, treated by cholecystectomy for microlithiasis. Ultrasound showed a pseudocyst in the body of the pancreas, 10 cm in diameter.

The patient was placed on total parenteral nutrition. Four weeks later she suffered severe diarrhea for four days. During this period, the abdominal mass became smaller coincident with a decrease in cyst diameter to 5 cm on ultrasonogram. No elevations of serum amylase were detected during hospitalization. Parenteral nutrition was suspended and the patient discharged. Total regression occurred on week 12 after diagnosis (Fig. 3).

Case 4. A 36-year-old man, with a history of two previous attacks of acute pancreatitis, entered the hospital with acute abdominal pain and a serum amylase level of 780 units. The pain started immediately after endoscopic retrograde cholangiopancreatography (ERCP). He was admitted with the diagnosis of acute relapsing pancreatitis. Eight days later, an epigastric mass of about 10 cm in diameter was palpable. Three days later, ultrasound showed a pancreatic pseudocyst of 12 cm in diameter and microlithiasis in the gallbladder. Total parenteral nutrition was started on day 8 following admission. Two weeks later, the patient experienced 48 hours of severe diarrhea, during which time initial regression of the abdominal mass was noted. Ultrasound now showed reduction of the pseudocyst to 5 cm in di-

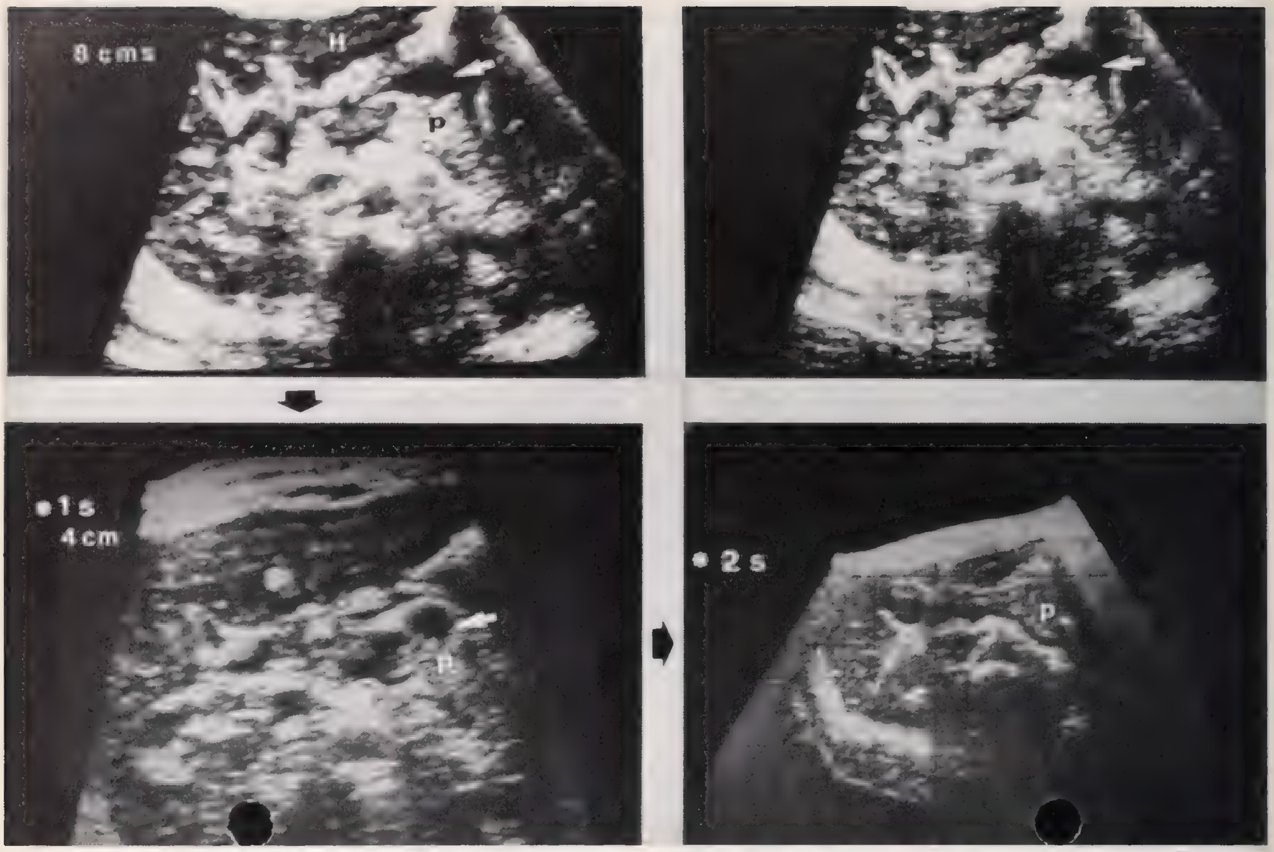
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Fig. 2. Case 2. Sonographic demonstration of resolution of pancreatic pseudocyst from 8 cm diameter to complete disappearance.

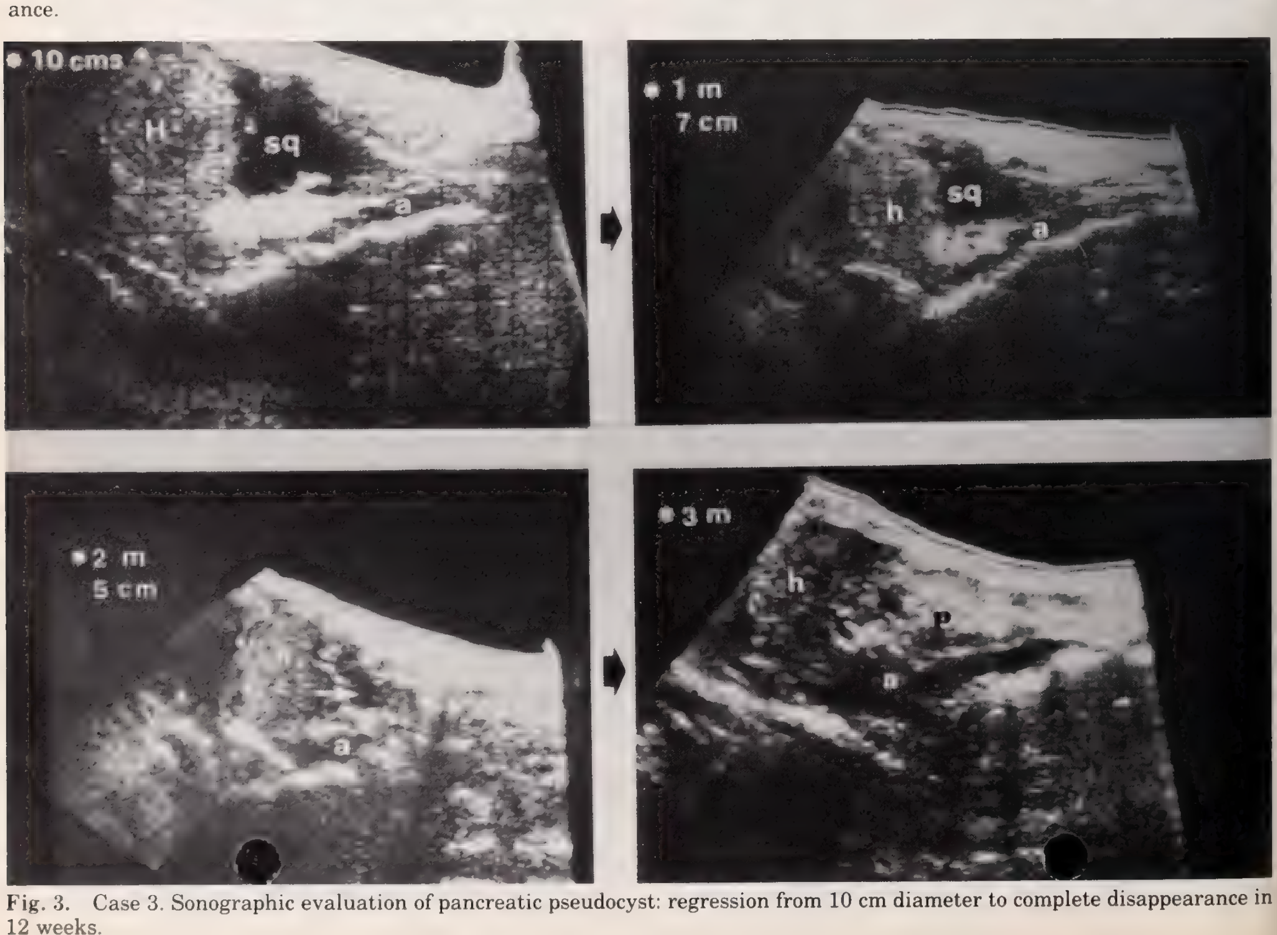
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Fig. 3. Case 3. Sonographic evaluation of pancreatic pseudocyst: regression from 10 cm diameter to complete disappearance in 12 weeks.

ameter (Fig. 4). The patient was discharged and followed in the clinic until total regression of his cyst was noted.

Case 5. A 65-year-old woman was admitted to the hospital with epigastric pain, vomiting, and a serum amylase level of 855 units. She was treated medically and her symptoms improved. Two weeks later a 15-cm epigastric mass was palpable. Ultrasonography showed a pancreatic pseudocyst, 12 cm in diameter. No abnormalities were noted in the gallbladder or biliary tree. The patient had no history of alcoholism or drug medication. Total parenteral nutrition was initiated.

The abdominal mass began to regress during the second week after diagnosis of the pseudocyst. Ultrasound now showed a 3-cm cyst (Fig. 5). One week later, the patient complained of acute abdominal pain in the left lower quadrant with fever, vomiting, and developed leukocytosis. A diagnosis of diverticulitis was made, and medical treatment with ampicillin, chloramphenicol, and tobramcin was started. Parenteral nutrition was maintained. After six days in which the patient did not respond to this regimen, exploratory studies revealed pelvic and perisigmoidal abscesses. Exploration of the upper abdomen revealed an abscess in the lesser sac, with partial pancreatic necrosis. Despite sigmoidectomy and drainage of all the abscesses, the patient died of sepsis.

Case 6. A 67-year-old man, a gypsy, appeared in the office accompanying his son who had returned for follow-up of chronic (15-year) recurrent pancreatitis. The father complained of minimal digestive complaints suggestive of peptic ulcer. Gastrointestinal series revealed a pressure defect in the posterior wall of the stomach suggestive of a pancreatic mass (Fig. 6), identified by sonography to be a pseudocyst (Fig. 7). A secretin test and gallbladder studies were normal. The blood amylase was in the 90s and remained at this level throughout. There was no fever, no leukocytosis, and, indeed, by the time investigations were complete, there were no gastrointestinal complaints. The patient was placed on a bland diet and followed with monthly sonography, blood amylase testing, and physical examination. In the ninth month following discovery of the cyst, it completely disappeared (Fig. 7).

Discussion

The earliest treatment of pancreatic pseudocysts was "marsupialization," that is, external

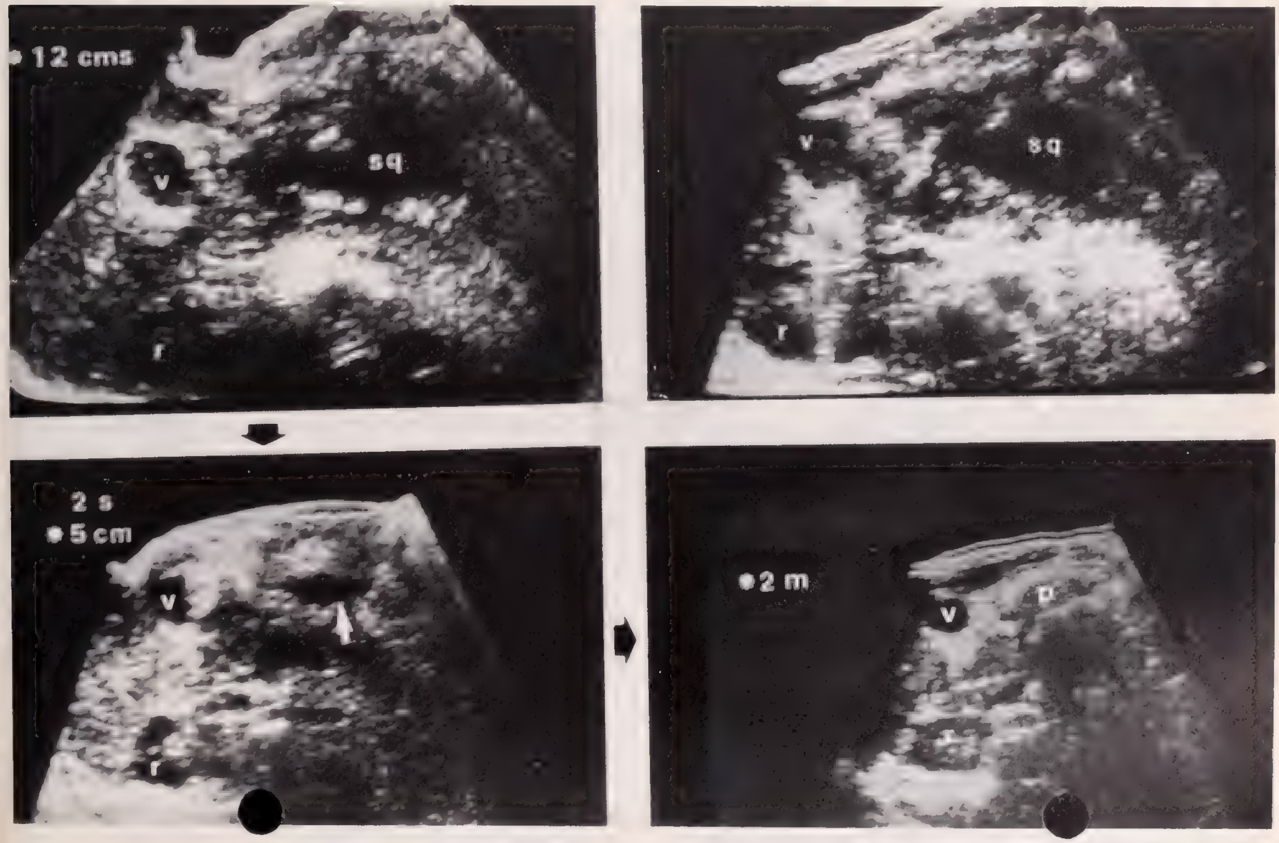

Fig. 4. Case 4. Sonographic demonstration of spontaneous resolution of a 12-cm pancreatic pseudocyst.

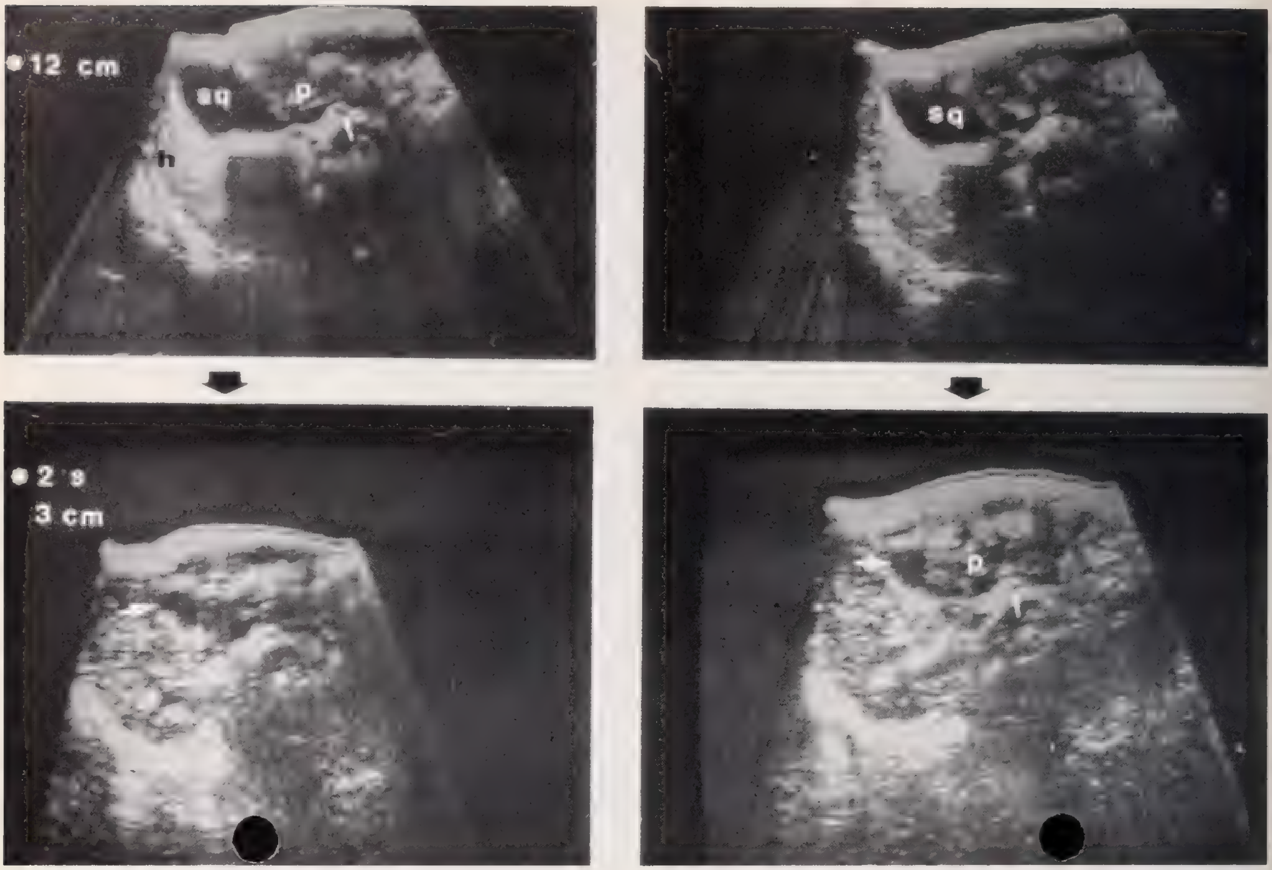

Fig. 5. Case 5. Ultrasound shows resolution of pancreatic pseudocyst from 12 cm to 3 cm.

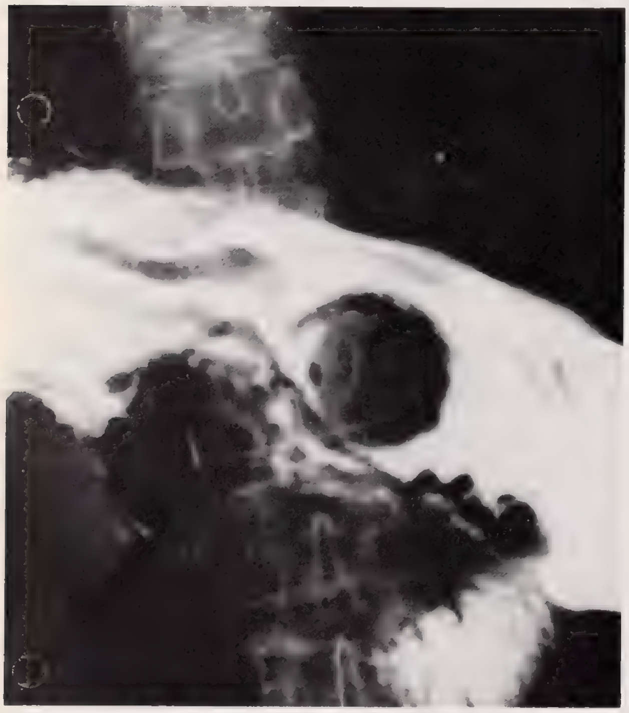

Fig. 6. Case 6. Gastrointestinal series showing compression of posterior wall of stomach by pancreatic pseudocyst.

drainage to avoid slow leakage, the development of pancreatic ascites, or precipitous rupture with consequent toxic shock. External drainage, of course, resulted in a troublesome pancreatic fistula with marked disturbances in fluid and electrolytes, frequent skin erosion by activated pancreatic ferments, wound infection, and, occasionally, wound disruption with evisceration. To avoid these complications, most surgeons delayed operations until the pseudocyst wall had matured and had become thick enough to permit internal drainage into a neighboring viscus, the stomach, duodenum, or jejunum by Roux-en-Y anastomosis. Although the results of internal drainage were generally satisfactory, there were complications of the procedure: marginal ulceration, anastomotic stenosis with cyst recurrence, and major hemorrhage due to arterial erosions.

Scanning techniques and operative endoscopy have provided additional therapeutic possibilities: percutaneous sonograph-guided needle aspiration and/or catheter placement for external drainage, and sonograph-guided endoscopic transgastric cyst puncture and drainage. These

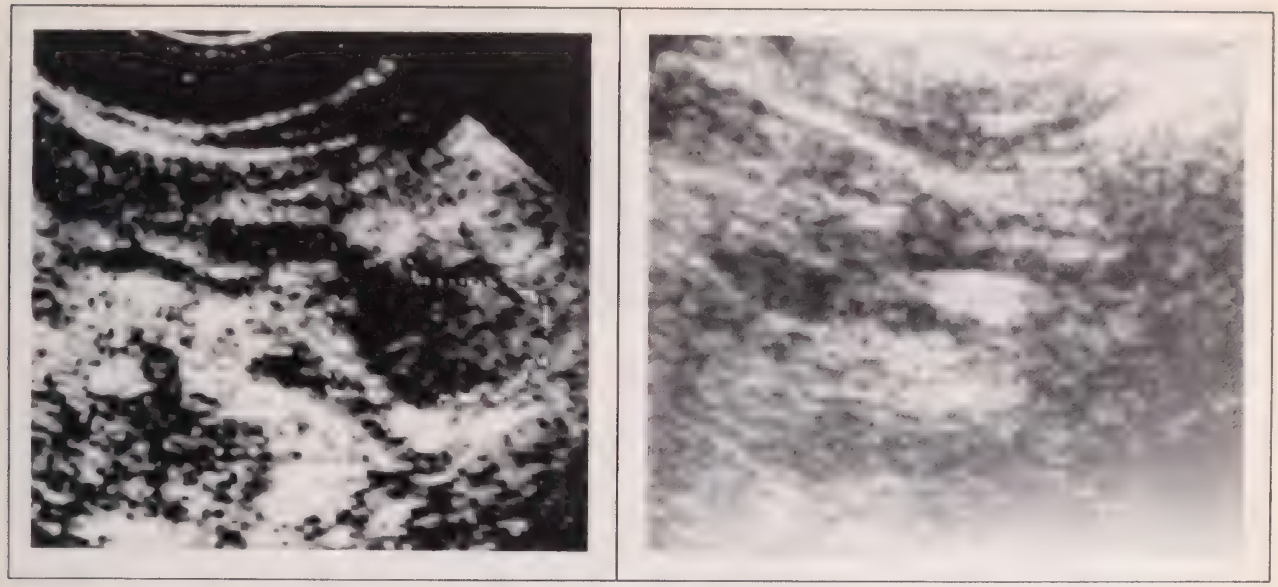

Fig. 7. Case 6. Sonographic view of resolution of pancreatic pseudocyst. **Left** Initial pseudocyst. **Right** Disappearance in 9th follow-up month.

techniques have been tried but not adequately tested to assess their efficacy. Moreover, sonography and computerized axial tomography (CAT scanning) have indicated that pseudocyst occurrence following pancreatic inflammation is far more frequent than hitherto suspected and that inflammation is far more frequent than hitherto suspected and that spontaneous resolution is not unusual (14).

The cases presented in this report demonstrate that pseudocysts as large as between 10 to 20 cm of diameter, with palpable masses and distortion of the gastrointestinal tract in barium studies, may regress spontaneously. Two cases were due to pancreatic trauma, blunt and operative injury; another two were due to cholelithiasis; and in two, the etiology was obscure. This contrasts with pseudocyst etiologies reported in most studies, in which the vast majority of the patients were alcoholics (2, 3).

Recent experimental studies in dogs have reported that a closed cavity, an actively secreting pancreas, and ductal communication with the cavity are necessary for the development of pancreatic pseudocysts in dogs (4). In that study, a canine pancreatic pseudocyst was produced by transecting the pancreas and implanting the two cut ends into a cavity formed six weeks earlier by placing a plastic bag intraperitoneally. In three animals with partial obstruction of the main duct, the pseudocysts were larger and persisted for more than a month. Thus, ductal obstruction appears to be a major factor in the pathogenesis of pseudocysts (15, 16). The failure of ERCP (17) and operative pancreatography (18) to visualize some cysts supports this hypothesis. Nakumara et al (19) have demonstrated plugs of precipitated protein in the pancreatic ductal system of patients with chronic pancreatitis. If the proteins plugs resolve or are dislodged or other causes of obstruction are released, the content of the cyst could drain through the ductal system itself. Silvis (17) presented two cases in which ERCP showed ductal communication with the cyst. These cysts resolved spontaneously within three and six months. In another laboratory study, Karlan et al (5) proved that when free drainage through an unobstructed pancreatic ductal system was permitted, the fistula and the space into which it drains tended to close spontaneously and the pseudocyst resolved. Is it possible that a pseudocyst with a wall of 1 or 2 or over 3 cm thick could collapse after it is emptied? It does seem possible, judging from a few reports of resolution of chronic percutaneous aspiration (20). In those cysts without intrinsic ductular obstructions, failure to resolve could be caused by a ball valve mechanism resulting from compression of the proximal ductal system by the cyst itself. Based on this hypothesis, an ERCP showing a patent communication between the main duct and the cyst could have prognostic value, that is, those patients in which such communication can be demonstrated have a good chance of spontaneous resolution and/or a low recurrence rate after percutaneous drainage. Unfortunately, in none of the reported series of percutaneous aspiration was this communication established before the procedure (20–22).

Spontaneous resolution of pancreatic pseudocysts has also been reported following erosion into a hollow viscus. This has been documented by radiologic studies and appears to occur within the first six weeks of cyst evolution, generally following a transient period of diarrhea (23). Two patients in this series displayed this phenomenon.

Disappearance of pancreatic pseudocysts can also occur by leakage or massive rupture into the peritoneal cavity. Massive rupture into free peritoneal cavity has occurred between 5% and 14% in different series (2, 3), followed by a mortality of between 30% and 40% (2, 3). Less dramatic leaking resulting in pancreatic ascitis was also reported by Sankaran and Walt (3) in 13% of their patients. Hemorrhage from the cyst and the upper G.I. tract have been seen in about 5% of patients reported, with a mortality of 60% (3). Abscess formation occurs in a frequency between 11% and 14%, and is associated with an extremely high mortality (2, 24). In Bradley's series (2), the overall complications in untreated pseudocyst was 41%. Twelve percent died as a result of complications. These complications occurred on an average of 14 weeks after the appearance of the pseudocyst.

The clinical and experimental data have resulted in different therapeutic approaches to pancreatic pseudocysts. Some, deeming the first six weeks to be a period relatively free of complications, advocate nonsurgical treatment in the hope of spontaneous resolution or at least maturation, that is, thickening of the capsule to make internal anastomosis more secure (20, 25). Others, concerned with the complication of rupture or abscess, prefer immediate exploration and external drainage (6). The procedures of percutaneous and endoscopic aspiration are still experimental.

In the series discussed here, spontaneous resolution occurred in all the patients reported. Disappearance of the cyst was observed two weeks to nine months following diagnosis. Treatment varied from total parenteral nutrition to simple bland diet. Conservative management initially should include:

no oral intake
parenteral alimentation
broad spectrum antibiotics
endoscopic pancreatography.

The first two measures "splint" the pancreas, reduce pancreatic secretion, combat nutritional deficiencies, correct metabolic deficiencies, restore metabolic balance, and have a beneficial effect on the underlying pancreatic inflammation (26–34). Antibiotics may inhibit secondary infection of the pseudocyst, preventing abscess formation. Pancreatography is useful in disclosing those cysts with ductal connection and might be therapeutic in dislodging protein plugs.

Conclusions

The clinical course of the patients studied and the experimental findings quoted lead to the suggestion of a possible nonsurgical therapy for pancreatic pseudocysts in patients satisfying the following criteria:

minimal or no symptoms
minimal or no elevation of serum amylase
absence of fever and leukocytosis
failure of the pseudocyst to enlarge as seen on successive sonograms.

Further, endoscopic pancreatography might be helpful in disclosing the pseudocysts likely to respond, i.e., those connected directly with the main duct. In fact, it is conceivable that the endoscopic delineation itself might be therapeutic in clearing proteinaceous plugs from the duct and/or reversing a ball-valve mechanism within the duct, preventing decompression.

Total parenteral alimentation and antibiotic coverage are suggested as important therapeutic measures. Transient diarrhea appears to herald sudden spontaneous disappearance of pseudocyst.

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The Value of Colonoscopy in the Diagnosis and Management of Severe Hematochezia

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Abstract

We reviewed 695 patients admitted to the hospital for gastrointestinal hemorrhage from 1979 to 1983. Of this total, 35 (5%) clearly had severe hematochezia, that is, bloody red stools, with a bleeding point distal to the ligament of Treitz. Thirty-one of these 35 patients needed a transfusion of at least 2 red cell units; the other 4 patients had hypovolemia and anemia. All 35 patients underwent colonoscopy (a total of 50 examinations). Precise diagnosis of the bleeding lesion by colonoscopy was made in 27 (77%). Bleeding diverticula and ulcers were the most frequent lesions. Thirteen patients were examined by angiography, which was diagnostic in 4 patients. Seven patients were treated endoscopically by monopolar coagulation. One perforation occurred. Nine patients were managed surgically; two complications occurred. No rebleeding occurred in any patient. We conclude that colonoscopy is a safe method of diagnosing the source of lower intestinal hemorrhage. Moreover, half of the 35 patients ceased to bleed, although the only treatment they received was supportive medical therapy.

The current literature on diagnosis and management of hematochezia emphasizes the role of angiography (1, 2). In particular, colonoscopy has rarely been considered an optimal method of diagnosing the source of acute rectal bleeding (3–7) because of technical problems related to insufficient and incomplete visualisation of the colon (5, 8).

The aim of our study is to stress the value of colonoscopy in the diagnosis of rectal bleeding and to evaluate the efficacy of endoscopic treatment of some specific lesions.

Material and Methods

We reviewed 695 patients admitted to hospital with the diagnosis of gastrointestinal hemorrhage between 1979 and 1983. Of these, 35 patients (5%) could be classed as having severe hematochezia (9). All had lesions located beyond the ligament of Treitz. In 31 the volume of blood loss required transfusion of at least two red-cell units and 4 had evidence of hypovolemia. The mean number of transfused red-cell units was 3.6 (range, 2 to 10). Twenty-two patients were men, thirteen were women. The mean age was 59.2, with a range from 28 years to 85 years.

Colonoscopy was performed in all patients (mean, 1.4 examinations per patient; total number of colonoscopies, 50), within the first hour of admission for patients passing fresh blood by rectum and within 24 hours for the others.

Colonoscopies. The colon was examined up to the ileocecal valve in 22 colonoscopies, to the hepatic flexture in 2, to the splenic flexure in 15, and to the ileum in 11.

The CFIB and more recently the CF_1TI Olympus fiberscopes were used; the latter instrument has a large 3.7 mm aspiration channel allowing adequate suction of fresh blood. Bowel lavage during the procedure was achieved with water injected manually through the endoscope. Bowel preparation was performed in 28 of the 50 colonoscopies; it consisted of oral lavage with the

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polyethylene-containing solution (10) in 18 cases, sometimes administered via a feeding tube, and salt-water enemas in 10 cases.

Angiography. Thirteen patients were examined angiographically, one before perioperative enteroscopy and 12 after colonoscopy. The indications for angiography in these 12 patients were negative colonoscopy (1 patient), lack of diagnosis after the first colonoscopy (8 patients), preoperative mapping of a lesion diagnosed endoscopically (2 patients), and confirmation of the endoscopic diagnosis (1 patient).

The mean time interval between endoscopy and angiography was 44 hours (range, 6 to 192). Mesenteric angiography was performed in 8 patients who were passing fresh bloody red stools.

Isotopic scan with technetium Tc 99m sulfur colloid was performed in 7 patients, 4 having negative colonoscopy, 2 having negative endoscopy and angiography, and 1 prior to other investigations. Six of the 7 scans were done while clinical signs of active bleeding were present. The mean time between endoscopy and scanning was 77 hours (range, 6–192 hours).

Results

Endoscopic diagnosis of the bleeding source was made in 27 of the 35 patients (Table 1). One perioperative ileojejunoscopy was performed in order to better define the extent of jejunal angiodysplasia diagnosed by angiography.

The most frequent bleeding lesions were ulcers and diverticula. All six diverticula were found in the right colon; active bleeding was observed in all (pulsated bleeding in 5, and oozing blood with fresh clot in 1).

Seven shallow ulcerations without any nodular pattern were found. In one of these patients, multiple ulcerations were seen in the ileum; other ulcers were single. Five were located in the left colon and one in the right. One ulcer was observed proximal to a sigmoid inflammatory diverticular stenosis. Two actively bleeding angiodysplasia were found, both in the right colon.

Angiography was performed in 13 patients, in one of the 13 before perioperative enteroscopy that confirmed the diagnosis of jejunal angiodysplasia, allowing precise delimitation of the lesion. The other 12 angiographies were done after colonoscopy. The absence of endoscopic diagnosis justified them in 1 patient in whom angiography was diagnostic and showed a bleeding source in the right colon which corresponded to a lymphoma found at surgery. In 8 patients, a negative first colonoscopy prompted the performance of angiography, which was not helpful to the diagnosis. Finally, angiography was performed after 3 diagnostic endoscopies. In 2 of these patients angiography was performed to allow precise localization of the lesion for the surgeon; one patient had extravasation of contrast in the right colon, which corresponded to a bleeding diverticula, and in the other patient angiography was needed to confirm the diagnosis (cecal angiodysplasia) made endoscopically. In the twelfth patient a doubtful left colonic bleeding was found by catheterization of the inferior mesenteric artery; two repeated colonoscopies of this patient had demonstrated a flow of blood up to the upper sigmoid from hemorroidal bleeding.

All 7 isotopic scans, although performed in 6 actively bleeding patients, were negative. Endoscopic management of active bleeding lesions was attempted in 7 patients: 2 angiodysplasia, 2 ulcers with oozing blood, 2 arterial hemorrhage on ileocolic anastomosis and 1 bleeding after the first polypectomy of a large rectal villous adenoma. In follow-up examinations ranging from 2 weeks to 3 months, no reoccurrence of bleeding was found in any patient.

TABLE 1

Endoscopic Diagnosis of Cause of Hematochezia (N = 27)

Diagnosis	No.
Ulcers	7
in colon	6
in ileum	1
Diverticula of colon	6
Colonic angiodysplasia	2
Ischemic colitis	2
Inflammatory bowel disease	2
Arterial bleeding on ileocolic anastomosis	2
Hemorrhoids	1
Protruded stoma	1
Jejunal angiodysplasia	1
Traumatic ulcer	1
Villous adenoma	1
Diffuse inflammatory anal bleeding	1

Discussion

Although colonoscopy actually plays a determining role in the diagnosis of lesions in the large bowel and even in the distal part of the ileum, it has rarely been considered an attractive method of diagnosis (3, 4, 7) or treatment of severe hematochezia. Recent literature emphasizes the use of angiography in diagnosing the source of rectal bleeding (1, 8).

Our series demonstrates a good diagnostic rate for colonoscopy in severe hematochezia

(77%). Moreover, endoscopic management was successful in 7 patients.

Polyethylene glycol solution (10), a new preparation administered orally or via feeding tube, could provide an adequate bowel cleansing system. The diagnostic efficacy of endoscopy depends on the endoscopist's training; recent technical developments, such as the large aspiration channel and superior optics, are helpful when blood clots are present or when preparation is poor. In this retrospective study, no specific criteria were used for choosing one of the 3 different types of bowel preparation; hence we cannot make valid inferences on this important problem. Our experience is that urgent total colonoscopy is technically feasible without any preparation in a patient actively passing fluid red blood by rectum.

Finally, a mobile intensive care team is essential for providing adequate cardiovascular monitoring during the diagnostic procedure.

As generally reported elsewhere (1–3, 6, 11, 12), bleeding diverticulum was one of the most frequently encountered lesions in our series (6 cases, all in the right colon). Surprisingly, bleeding ulcerations in our series were as frequent as the diverticula. This lesion has not been described previously in the colon, except experimentally (13). However, bleeding ulcers have been reported in the small bowel by Norman et al (2), and one patient in our series had the same type of lesion in the ileum. The majority of the bleeding ulcers in our series were located in the left colon. Their endoscopic appearance was not comparable with ulcerations encountered in inflammatory bowel disease or infectious colitis.

The actively bleeding angiodysplasia in the right colon in our series (2 patients) is in accord with earlier studies (1, 14–16), as is the fact that the 2 patients in whom it occurred were elderly (1, 12, 14).

Although the effectiveness of angiography is underlined in the literature (1, 8), in our series diagnosis was obtained in only 4 of the 13 angiographs, 2 of them performed after colonoscopic localization of the bleeding. Among 8 patients bleeding actively at the time of angiography, only one case of blood extravasation was seen. The value of angiography in the absence of blood extravasation is questionable, since angiodysplasia may coexist with diverticulosis in the same patient and can be misinterpreted as the source of bleeding.

Endoscopic management of hematochezia was attempted in 7 patients in our series. Two bleeding angiodysplasia were coagulated in the right colon. The efficacy of this method has been previously reported (13, 16). Moreover, 2 single colonic ulcers were treated. Although the pathogenesis of such lesions is not always known (17), association with ischemic damage is likely. One patient had a perforation some days after endoscopic treatment, which proved fatal. The cause of death was confirmed by postmortem examination, which showed lesions of ischemic colitis at the electrocoagulation site. Jensen et al have indeed recently observed that delayed perforation in canine colon treated with the instrument of monopolar design (13) and viscous distention during endoscopy significantly increases the incidence of transmural thermal injury (18). Furthermore, the thickness of the bowel wall must be considered in deciding on the coagulation mode (13); even use of Argon laser photocoagulation or bipolar electrocoagulation must be considered with caution in cases of bleeding colonic ulcers.

TABLE 2

Indications for Surgery in 35 Patients with Hematochezia (N = 9)

Condition	No. of patients
Diverticula	4
Jejuno-ileal angiodysplasia	1
Hemorrhoids	1
Ischemic colitis	1
Protruded stoma	1
Sigmoid diverticulitis with ulceration	1

Hemorrhage after endoscopic resection of large polyps is a well-known complication. Electrocoagulation helped stop the bleeding in the patient with postpolypectomy hemorrhage in our series.

Finally, we succeeded in controlling two active arterial bleedings on ileocolic anastomosis, which persisted in spite of supportive medical therapy.

Eleven patients were surgically managed; about half of them had hemicolectomy for bleeding diverticula. However, surgery should never be considered without precise preoperative diagnosis. This prevents unnecessary bowel resection. In some specific cases, perioperative endoscopy of the small bowel can provide precise diagnosis. Of the 35 patients in our series, 19 had no endoscopic or surgical treatment. Of these, 8 were undiagnosed; 7 had diffuse inflammatory lesions, some of which were ulcerated; one had an iatrogenic rectal ulcer; another had anal bleeding; and two who were poor surgical risks had diverticula.

TABLE 3

Diagnosis of 19 Untreated Patients among 35 Patients with Hematochezia

Diagnosis	No. of patients
Undiagnosed	8
Ulcers	4
Inflammatory bowel disease	2
Diverticula	2
Traumatic ulcer	1
Ischemic colitis	1
Diffuse anal bleeding	1

Based on this retrospective study, we suggest the following diagnostic procedure in cases of severe hematochezia:

- Since nasogastric aspiration could miss a bleeding duodenal ulcer, it is important to start investigation by upper gastrointestinal endoscopy.
- If blood is not flowing from the upper digestive tract, we perform a colonoscopy without bowel preparation. In the absence of a diagnosis, one must consider two possibilities: either the bleeding persists and the patient will undergo angiography, or the bleeding stops and a second colonoscopy will be performed after bowel preparation, administered orally or—if needed—via feeding tube.
- If the latter procedure fails to yield a diagnosis, angiography combined with isotopic scan are indicated.

The low efficiency of the isotopic scan in our series contrasts with the data reported by Abass et al (19). Indeed, their results indicate a good diagnostic rate of technetium Tc 99m sulfur colloid isotopic scan compared to angiography, even when the bleeding rate is low. The reason for these conflicting results may be an inadequate definition of what constitutes "active bleeding."

Endoscopic treatment should be reserved for actively bleeding angiodysplasia. There is no controlled data suggesting that other colonic lesions must be treated in the same way. Moreover, it must be stressed that about half our patients had only supportive medical therapy. Further controlled clinical trials are needed to compare the value of colonoscopy and angiography in diagnosing the exact source of severe lower intestinal bleeding.

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Vascular Pancreatitis: A Neglected Disorder

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Much has been written concerning the vascular complications of acute pancreatitis, but relatively little about the vascular etiologies of pancreatic inflammation.

The vascular complications of acute pancreatitis derive from:

increased bleeding tendencies
increased blood coagulability (1–3).
blood volume deficits
increased vascular permeability (4)
arterial and venous erosions (5–7)
decreased parenchymal perfusion (8)

The hemorrhagic diathesis in pancreatitis is complex and results in hemorrhage (5, 6) locally into the pancreas, retroperitoneally, intraperitoneally (ascitic effusion), intrathoracically (pleural effusion), loculated (lesser sac), metastatically (ovarian cyst), or distantly—Cullen (9) and Grey Turner (10).

The bleeding is due to increased capillary stasis, increased capillar permeability, increased blood proteolytic activity, or vascular disruption by activated pancreatic ferments. These alterations result from massive release from the pancreas of activated trypsin (11), activated elastase (12–15), and bradykinin (16). Bradykinin locally induces precapillary arteriolar constriction, which results in capillary bed stasis and parenchymal ischemia. Bradykinin systemically evokes a showering effect activating the hallinogen, thrombin, and liposymal systems.

The hypercoagulability observed in pancreatitis also results from increased extrapancreatic activated pancreatic ferment activity: trypsinogen activated to trypsin increases release of thrombin, which in turn increases fibrinogen conversion to fibrin. In the more advanced inflammations fibrin and fibrinogen may be degraded, and this fibrinogen depletion coupled with intravascular platelet aggregation results in a disseminated intravascular coagulation syndrome.

In the past the hemorrhagic dyscrasia of acute pancreatitis could only be revealed by alterations in antithrombin and antiproteolytic titers. Now the defects can be measured more directly and qualitatively by radioimmune measurement of blood trypsin (17), serum fibrinogen levels, serum fibrinogen consumption, and so on.

The serious question arises as to whether administration of heparin (18–20) during acute pancreatitis might have therapeutic value, since in animal experiments heparin is antagonistic and inhibits the hematologic alterations.

Major hemorrhage in acute pancreatitis presents an acute surgical emergency because of digestive vascular erosions. The splenic artery and vein are at greatest risk because anatomically they are engulfed by pancreatic parenchyma. However, any pancreatic blood vessel, especially those encroached upon by developing pseudocysts, is in jeopardy. Indeed, disruption of peripheral vessels may occur, resulting in metastatic cutaneous hemorrhage.

Shock, the most serious vascular complication of acute pancreatitis, results from a combination of (a) decreased oxygenation due to surfactant depletion (21), atelectasis (22), pleural effusion (23), or pulmonary edema (24); (b) decreased cardiac function due to cardiac depressant factor (25) or pericardial effusions (26); and (c) blood

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volume deficit due to hemorrhage (27, 28), capillary status and permeability (29), or vascular effusions and sequestration.

Recognition of the vascular complication of acute pancreatitis and the development of appropriate therapeutic measures will continue to lower the subsequent morbidity and early mortality of pancreatic inflammation. The administration of steroids (30) and heparinization (18–20) await controlled clinical trials.

On the other side of the coin are vascular-induced pancreatic inflammations. The pathogenetic mechanism involved in vascular pancreatitis is acinar cell anoxia, resulting in disruption of lysosomal vacules, mitochondria, and enzyme granules. Liberated lysosymes activate intracellular pancreatic ferments and initiate autocellular digestion.

Experimentally, human vascular pancreatitis has been simulated by partial pancreatic arterial ligation (31), pancreatic venous thrombosis (32), and isolated duodenal loop (33). In the latter protocol, the severity of pathology could be correlated with the degree of vascular occlusion (34, 35). The pathogenetic sequence was as follows (36): pancreatic edema, leading to arterial compression and venous thrombosis, resulting in parenchymal ischemia, causing cellular breakdown, fat necrosis, and interstitial hemorrhage.

The clinical varieties of vascular pancreatitis include:

diffuse pancreatic atrophy
disseminated focal pancreatic fibrosis
disseminated focal pancreatic necrosis
pancreatic apoplexy
diabetic pancreatitis
low-flow syndromes of induced pancreatitis
periarteritis nodosa pancreatitis (37)
lupus erythematosus pancreatitis (38)

The first five varieties occur in the aged and in diabetics. These lesions have been amply demonstrated in autopsy material. The extent of the abnormality can be correlated with the degree of arterial and arteriolar sclerosis, vascular lesions that occur at a much earlier age in diabetics.

Pancreatitis occurring in low-flow syndromes is seen in patients with:

hypovolemic and toxic shock (28, 39, 40)
myocardial infarction
severe diabetic ketoacidosis
Ortner syndrome—abdominal angina
abdominothoracic aortic aneurysms (40)
renal transplantation (41)

In all these forms there is a shunting of blood from the abdominal viscera, a process that results in greatest deprivation of flow to the pancreas. The resultant ischemia induces acute pancreatitis. Indeed, pancreatic necrosis is often the unsuspected and undocumented node of exitus.

An understanding of the vascular etiologies of pancreatic inflammation is important to the physician, for it indicates possible therapeutic prophylactic measures:

avoidance of blood volume deficits
vigorous treatment of shock
avoidance of long periods of hypotension
surgical correction of vascular obstructions.

For geriatric and diabetic patients, therapies must be developed to prevent the development of arteriosclerosis, a major concern of modern society.

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Bronchopleural Fistula: Pulmonary Changes in a Neonate

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During the treatment of respiratory distress and pneumothorax in preterm infants, a continuous and massive air leak through a bronchopleural fistula may occur. Invariably, this is traumatic in origin, being produced either by an endotracheal suction catheter or by a thoracotomy tube (1–4). The fistulous tract forms a path of least resistance for the inspired air; consequently, further alterations in pulmonary ventilation, as well as morphologic changes, will occur. We report here on a neonate with a bronchopleural fistula caused by an endotracheal suction catheter, in whom pulmonary pathologic changes reflecting altered airflow were observed.

Case Report. A 900-gm infant, of 28 weeks' gestation, was transported to the neonatal intensive care unit at 6 hours of age. The 30-year-old, gravida 5, para 4003 mother required a cesarean section for premature labor with ruptured membranes. The Apgar scores were 1 and 4 at one and five minutes, respectively. The newborn required ventilatory support with a volume-cycled Bourn's LS 104 150 ventilator. During the first seven days, the infant had three double-volume exchange transfusions for hyperbilirubinemia. On day 7, a large amount of frank blood was suctioned from the endotracheal tube. Thirty minutes later, the infant had seizures, turned dusky red and bradycardic, and became unresponsive to tactile stimulation. A transcutaneous oxygen monitor showed rapid decline in cutaneous $TcPO_2$ from 60 Torr to 1 Torr. During resuscitation, copious blood-stained secretions were suctioned from the trachea. A tension pneumothorax was detected by high-intensity fiberoptic transillumination and confirmed by x ray. A bronchopleural fistula was suspected when it was not possible to evacuate the pneumothorax after three chest tubes were placed in the right pleural cavity. Attempts at selective intubation of the left main bronchus were unsuccessful. Mechanical ventilation with an FIO_2 of 1.00 was continuously used until the patient's death on day 11, when he developed seizures, a tense fontanel, bradycardia, hypotension, and acidosis.

At autopsy, the infant was edematous and weighed 1,260 g. The skin was blotchy red and the lower extremities were cyanotic. The right chest had three thoracostomy incisions and two chest tubes in place. Both lungs were atelectatic. Neither a pneumothorax nor pneumomediastinium was demonstrated. The right lung was congested, and hemorrhagic fibrinous adhesions were present in the interlobar fissures, on the parietal pleura, and around the chest tubes. A subpleural cyst was present on the right diaphragmatic surface (Fig. 1). The right mainstem and lower lobe bronchial mucosas were ulcerated, and showed squamous metaplasia of the epithelium and fibrosis of the walls. The anterobasal segmental bronchus showed cylindrical dilatation extending to the pleural surface and terminating in the subpleural cyst. It fit a no. 5 Fr Argyle suction catheter (Argyle; Division of Sherwood Medical, St. Louis, MO 63103) passed through a 3.0-cm Portex uncuffed endotracheal tube (Portex, Wilmington, MA 01887), which was used to provide airway in the infant.

The perforation was reconstructed (Fig. 2). Microscopically, the fistula consisted of a tract lined by fibroblasts, granulation tissue, and

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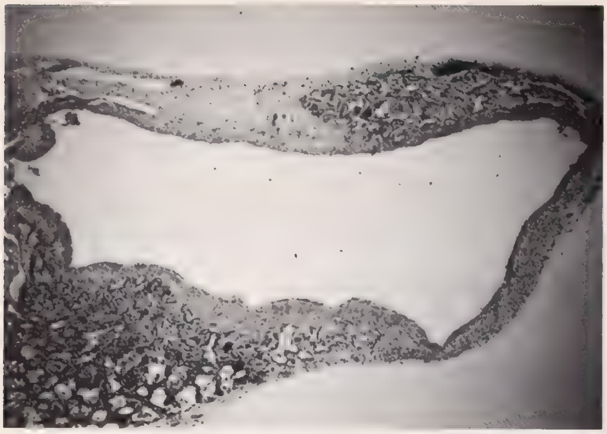

Fig. 1. Above Large subpleural cyst on diaphragmatic surface of right lung (hematoxylin and eosin, ×20, original magnification).

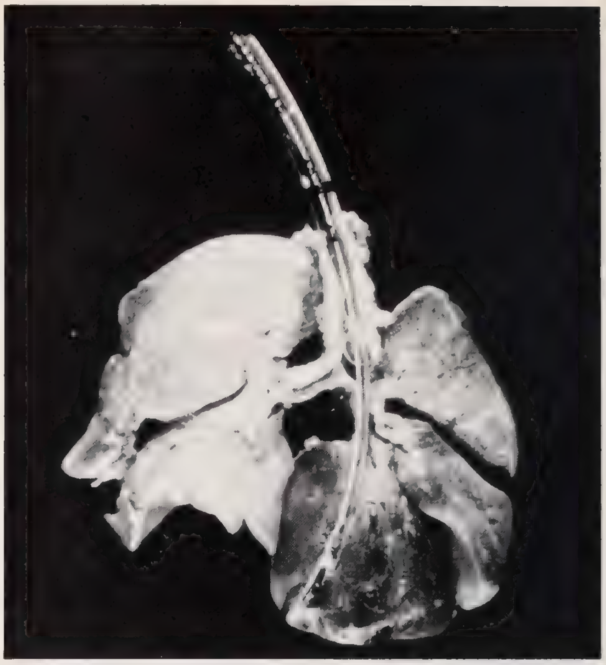

Fig. 2. Right Reconstruction of perforation: suction catheter, inside endotracheal tube, passes into right-lower-lobe bronchus, through anterobasal bronchus, and terminates in subpleural cyst.

foamy macrophages in continuity with the proximal segmental bronchus. Adjacent lung showed mucus retention in airways, irregular alveolar distention, focal parenchymal hemorrhage, and fibrosis (Figs. 3, 4). The remaining four pulmonary lobes showed mild changes of bronchopulmonary dysplasia with focal alveolar fibrosis, and muscular hypertrophy of terminal bronchioles and arteries (Fig. 5). Elsewhere, there were hepatic hematomas and gastric erosions. Death was due to massive subependymal and intraventricular hemorrhage.

Discussion. Iatrogenic disease is an important cause of morbidity and mortality in neonatal intensive-care nurseries (5). In this 900-gm infant of 28 weeks' gestation who was mechanically ventilated for 11 days, bronchopleural fistula, traumatic injury of the right mainstem bronchus, and bronchopulmonary dysplasia were treatment-related injuries of the respiratory tract.

In patients who undergo mechanical ventilation, periodic physiotherapy and care of the endotracheal tube by saline lavage and suction are standard procedures to maintain a patent airway. Since pulmonary air leaks commonly occur in such infants (5), perforation of a bronchus by a suction catheter as the cause of pneumothorax may go undetected, even at autopsy, unless carefully searched for. Clinical signs that indicate the development of a bronchopleural fistula are sudden clinical deterioration following suction of the endotracheal tube, recurrent pneumothorax despite the use of multiple chest tubes, and blood-stained endotracheal aspirates (1–3). The diagnosis of iatrogenic bronchopleural fistula may be confirmed by radiologic contrast study of the tracheobronchial tree.

When the site of perforation in the pleura is localized, it may be closed with sutures. Anderson and Chandra (1) have shown that premature lung may easily be perforated by a suction catheter that is inserted beyond a safe length (oral—carinal length), which is directly proportional to the infant's weight. Greco and associates (7) describe three infants with bronchopulmonary dysplasia who developed "bronchiectasis" in the lower lobes. Their description of "greatly dilated bronchi communicating with subpleural blebs" is similar to our finding (Fig. 1) and may well have the same etiology. Since the perforation most often occurs at the anterobasal and medial-basal segment of the right lower lobe (1), such areas should be closely examined at postmortem examination of infants with recurrent pneumothorax.

The changes in the right mainstem bronchus in this patient are suggestive of traumatic injury by an endotracheal tube advanced beyond the carina. The endotracheal tube in such a position may have facilitated the entrance of the suction catheter into the right-lower-lobe bronchus. As examination of all chest x rays showed good placement of the endotracheal tube, the injury must have occurred during attempts at intubation or during airway suction. Potential late se-

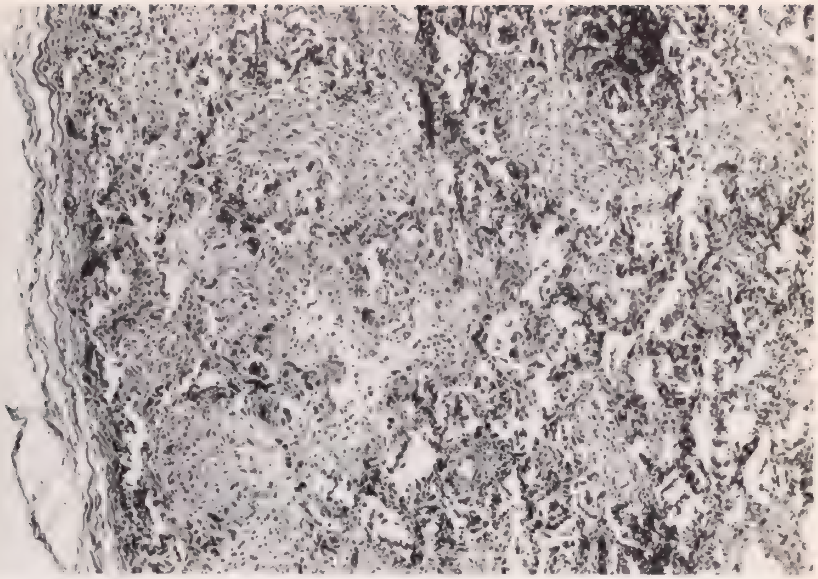

Fig. 3. Right lower lobe of lung showing parenchymal hemorrhage, consolidation and fibrosis, and pleural adhesions (hematoxylin and eosin, ×40, original magnification).

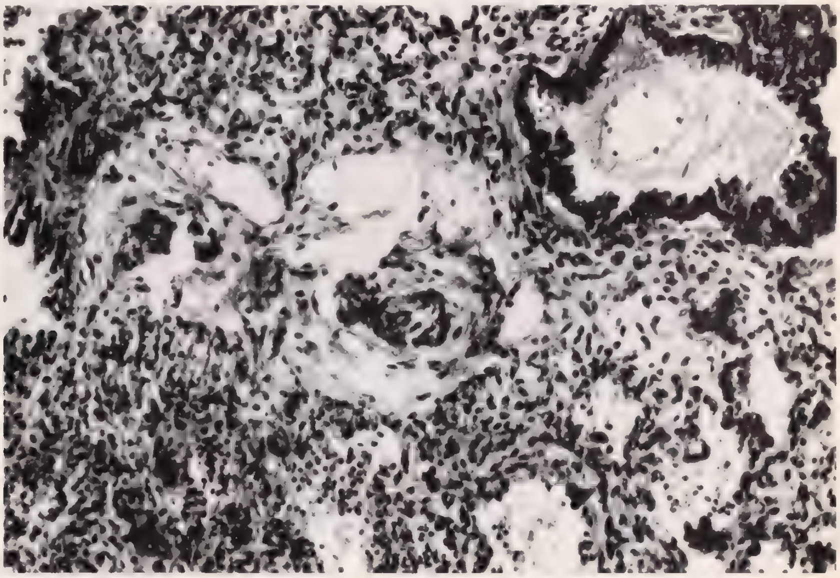

Fig. 4. Right lower lobe of lung showing mucus in airway, congestion, and fibrosis (hematoxylin and eosin ×200, original magnification).

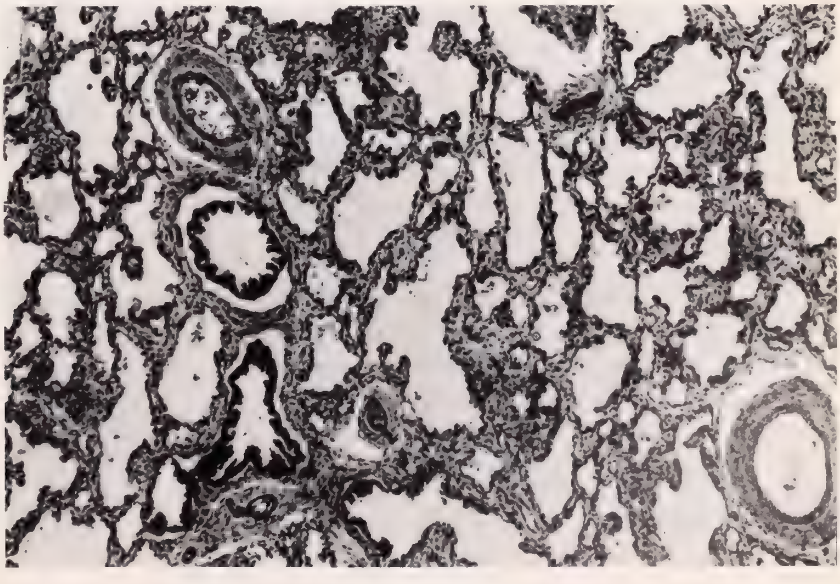

Fig. 5. Left lung showing interstitial thickening and muscular hypertrophy of vessels and airways (hematoxylin and eosin ×100, original magnification).

quelae of injury to the mainstem bronchus are lobar emphysema, recurrent pneumonia, and atelectasis (6).

Considering that this infant had received mechanical ventilation at the frequency of 30–40 cycles/min with a peak pressure of 25 cm H_2O and PEEP of 4 cm H_2O with FIO_2 of 1.00 for more than 5 days, the degree of bronchopulmonary dysplasia is unexpectedly mild (8). The mild pulmonary changes correlate with the benign clinical course prior to the lung perforation. Subsequently, a large fraction of the tidal volume supplied by the ventilator escaped through the bronchopleural fistula in the right lower lobe (Figs. 3, 4), and the remaining lobes were relatively protected from the effects of high oxygen concentration and barotrauma. The marked fibroblastic proliferation of the fistulous tract manifests the oxygen effect on pulmonary parenchyma.

Iatrogenic bronchopleural fistula should be recognized and treated promptly. More important, such injuries should be prevented by careful suction techniques. Because perforation occurs beyond the carina, the length of the suction catheter should not exceed more than 1 cm of the length of the endotracheal tube (1). Measuring and marking the suction catheter before insertion should be part of the standard endotracheal suction procedure. Moreover, rotating the catheter and pulling it back while applying suction prevents adhesion of tracheal mucosa to the catheter orifice and minimizes the risk of mucosal tears.

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Pneumonia Presenting as a Radiographic Mass in a Child

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A variety of pathogens may cause parenchymal pulmonary disease in children. Radiologic manifestations are also variable, and it has been shown that radiologic appearances are not reliable predictors of the causative agent (1). Nevertheless, the alveolar and interstitial infiltrates of acute inflammatory disease are rarely confused with noninflammatory processes in children without underlying chronic pulmonary disease. Primary or metastatic neoplasms rarely present a problem in differential diagnosis, mainly because neoplasms have a different clinical presentation, the radiographic features of a mass, and relatively low frequency among young patients. Less well known is the possibility of acute pneumonia presenting as a radiographic mass in children as exemplified in the following case report.

Case Report. A 10-year-old girl was admitted to hospital with a one-day history of fever, vomiting, and sore throat. Her temperature was 39.3°C and physical examination yielded evidence of conjunctivitis and pharyngitis. There were no abnormal findings from the chest; specifically, the lungs were clear on auscultation. The spleen was palpable about 2 cm below the left costal margin. The white blood count was 24,000 per cu/mm with 63% polymorphonuclear leukocytes and 24% band forms. The sedimentation rate was 75 mm/hr. Urinalysis revealed albuminuria, ketonuria, and 1–2 white and 2–3 red blood cells per high-power field. All other laboratory values were within normal limits.

Radiographic examination of the chest revealed a well-circumscribed round density in the left lower lobe, with features of a pleural-based posterior intrapulmonary mass (Fig. 1).

Despite the strong clinical evidence of an infection, the paucity of localizing physical signs and the radiographic appearance raised suspicion of a tumor unrelated to the symptoms and sufficient alarm to result in consultation for computed tomography and other imaging studies.

The radiologists' impression was that the most likely explanation was a pneumonia of the rare configuration described as a "spherical pneumonia." This explanation was accepted reluctantly and the patient was treated with ampicillin (1.5 gm/day). She became afebrile within 2 days, and a second chest roentgenogram 5 days later showed marked resolution of her "mass" (Fig. 2). The throat culture revealed heavy growth of Group A beta hemolytic streptococcus. Blood and urine cultures showed no growth and cold agglutinin titers were negative.

Discussion. Round or "spherical" pneumonias in children have been reported by a number of authors in the radiologic literature (2, 3). These pneumonias are rare, which accounts for the lack of their recognition and the alarming conclusion often arrived at, that they represent tumors (2). Their rarity is surprising considering the extensive pathologic and radiologic experimental evidence that the inflammatory exudate of pneumococcal infection tends to spread centrifugally through interalveolar communications, largely through the pores of Kohn and channels of Lambert, and without regard for segmental boundaries (2–4).

Most round pneumonias in children are caused by *Streptococcus pneumoniae,* although no organism has been identified in many cases which have responded to antibiotics. It is questionable whether our patient had beta hemolytic streptococcal pneumonia. The majority of round pneumonias (19 of 22 in the series of Rose and Ward) (2) are located in posterior segments of either lung. Symptoms and signs of an infection

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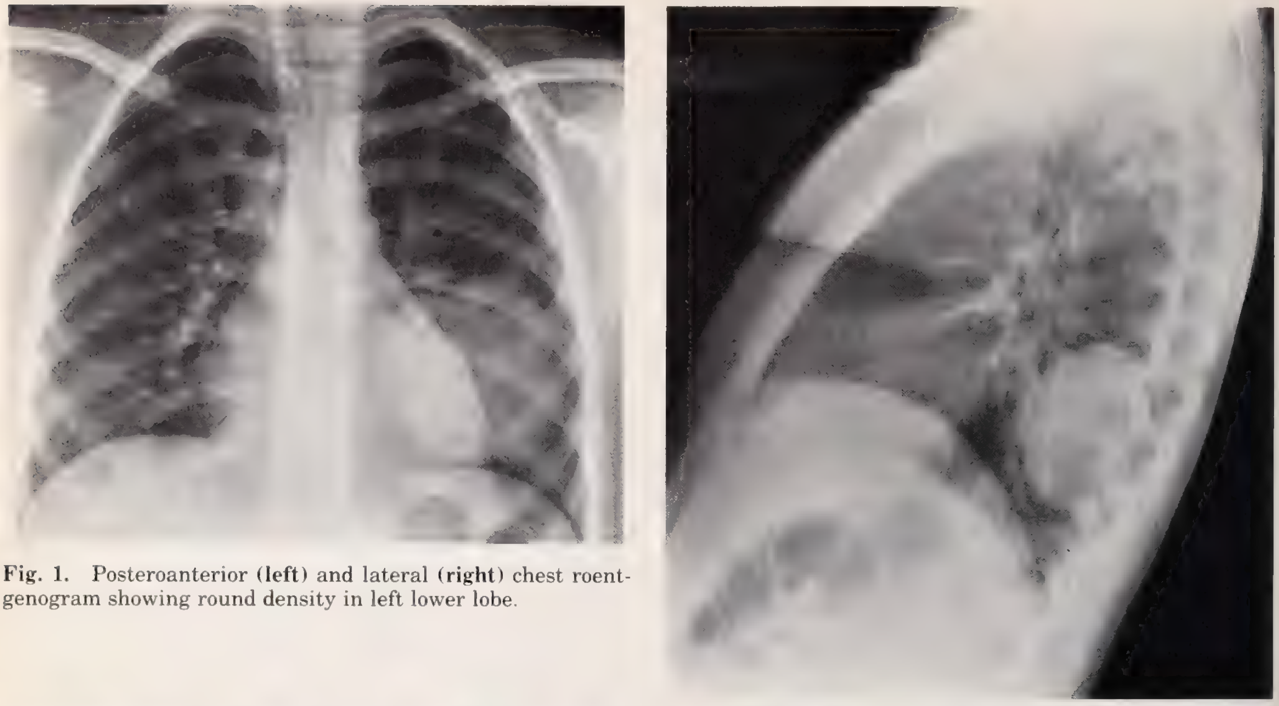

Fig. 1. Posteroanterior (**left**) and lateral (**right**) chest roentgenogram showing round density in left lower lobe.

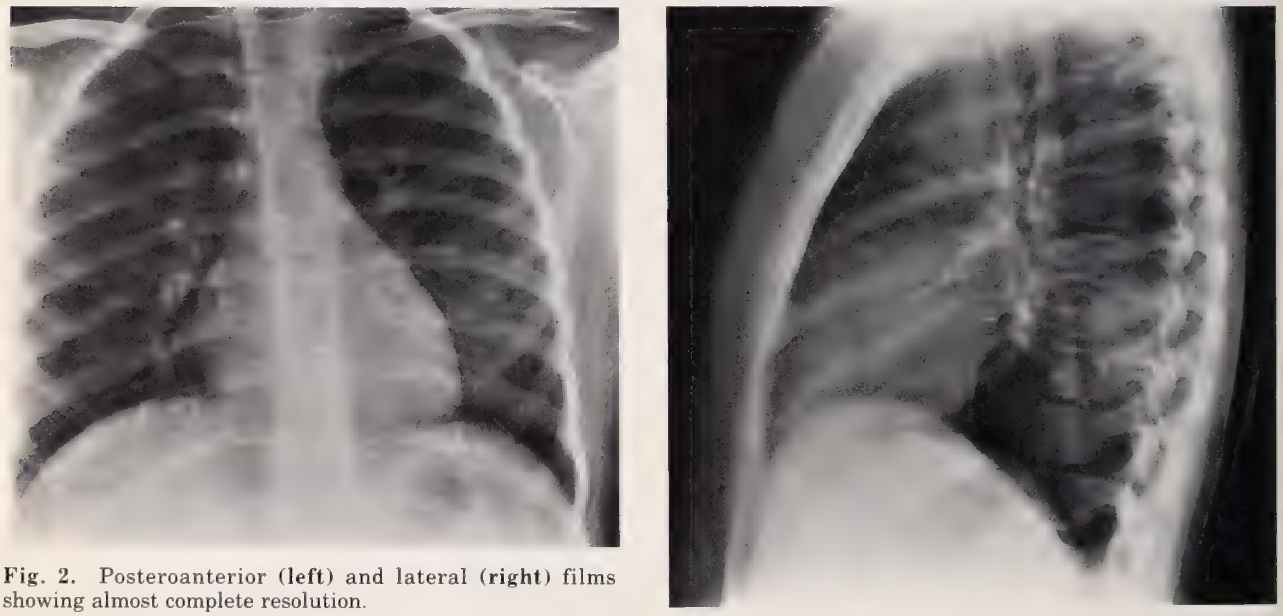

Fig. 2. Posteroanterior (**left**) and lateral (**right**) films showing almost complete resolution.

have always been present (2, 3). Despite these symptoms and signs, fear of an underlying pulmonary or mediastinal neoplastic process has dictated extensive investigation in some patients (2), and the temptation may prove more and more difficult to resist with modern-day imaging technology. I suggest that the presence of a radiographic mass involving the lung or mediastinum in a febrile child, especially if associated with signs of respiratory disease, should be initially treated as a bacterial pneumonia. Further investigation is necessary only if the mass fails to resolve after appropriate antibiotic therapy.

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Spontaneous Pneumomediastinum Following Inhalation of Alkaloidal Cocaine and Emesis: Case Report and Review

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Pneumomediastinum, also known as mediastinal emphysema, has been recognized since 1819, when it was accurately described by Laennec (1). Causes include penetrating injuries of the neck and chest, radical neck dissection, tonsillectomy, dental procedures, tracheostomy, and perforations of the esophagus, upper airways, or, rarely, stomach or bowel (2–4). It is, however, not generally appreciated that pneumomediastinum may develop without any apparent cause (2, 4).

The spontaneous occurrence of air in the mediastinum, although rare, is a well-described entity (2, 3, 5). Pneumomediastinum arising either spontaneously or due to underlying pulmonary disease has been called "medical mediastinal emphysema" by Bodey (2), who reported an incidence of approximately 1 in 40,000 hospital admissions. More recent studies, however, suggest the incidence to be greater (3).

Spontaneous pneumomediastinum, usually a benign condition, can be indistinguishable clinically from potentially life-threatening causes of air in the mediastinum, such as esophageal perforation (6, 7).

We present a case of spontaneous pneumomediastinum following prolonged inhalation of "free-based" cocaine and emesis. The pathophysiology, clinical course, treatment, and complications of spontaneous pneumomediastinum are briefly discussed.

Case Report. A 23-year-old man came to the emergency room with substernal, epigastric, and neck pain. The patient was in good health until the evening prior to admission when, after several hours of inhaling alkaloidal cocaine, he developed nausea and vomited, with retching. Upon awaking the next morning, he noted severe, sharp, constant substernal, epigastric, and neck pain, which was exacerbated by swallowing, deep respiration, and neck movement. The pain radiated to the mediastinum with neck movement. The patient reported no hematemesis, hemoptysis, dyspnea, cough, pulmonary disease, trauma, or tobacco or alcohol abuse. His medical history was significant for gastritis, treated with cimetidine, and occasional episodes of upper abdominal pain.

On physical examination, the patient was anxious, but not in acute distress. He was afebrile, with a respiratory rate of 22 breaths per minute. Slight crepitus was palpated in the anterior neck bilaterally. The chest was clear to auscultation. Cardiac examination was normal. Abdominal examination revealed mild tenderness to deep palpation in the epigastrium, but was otherwise within normal limits.

Laboratory analysis showed a white blood cell count of 13,500, with 10 bands. Serum electrolytes, hematocrit, urinalysis, liver function

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tests, amylase, and arterial blood gas analysis were within normal limits. Chest x rays revealed air in the mediastinum and subcutaneous tissues of the neck, without pneumopericardium, pneumothorax, or pleural effusion (Fig.). Anterior-posterior and lateral neck films also demonstrated subcutaneous emphysema. A nonspecific gas pattern, without evidence of free air, was noted on the abdominal radiographs. The electrocardiogram was normal.

The patient immediately underwent an esophagram, which was normal. The patient was admitted, started on intravenous antibiotics, and carefully observed. He was permitted nothing by mouth.

The patient rapidly improved. The leukocytosis resolved by the second hospital day. Serial chest x rays showed rapid resolution of the pneumomediastinum. Antibiotics were discontinued on the fourth day, and the patient was fed. He was discharged on the sixth day, with complete resolution of his symptoms, pneumomediastinum, and subcutaneous emphysema.

Discussion. Medical mediastinal emphysema usually occurs in relatively young, healthy individuals following acute, intermittent respiratory obstruction. Underlying pulmonary disease, most commonly asthma, pertussis, or pneumonia, was found in less than one-half of all cases reported (3). Munsell (8) noted that approximately one-half of the early cases reported were related to parturition. Mattox (9), in a large series of patients with pneumomediastinum, found that only 3% had truly "spontaneous" pneumomediastinum. These patients were heavy marijuana smokers or intravenous heroin users who had performed extended Valsalva maneuvers.

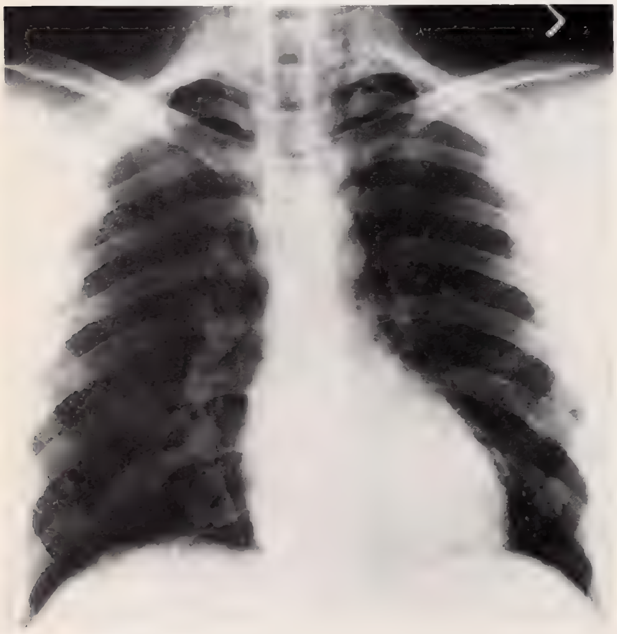

Fig. Posteroanterior chest x ray. Note pneumomediastinum and subcutaneous air in neck.

The extended Valsalva maneuver has been implicated as the cause of spontaneous pneumomediastinum (4, 8, 9). Prolonged Valsalva maneuvers result in acute, intermittent lower-airway obstruction, greatly increased intraalveolar pressure, and subsequent rupture of alveoli. Spontaneous pneumomediastinum has been reported following emesis, straining, defecation, weight lifting, sneezing, parturition, and marijuana smoking (2–4, 8, 9). Prior to this report, three cases of pneumomediastinum following inhalation of alkaloidal cocaine were reported, also presumably secondary to prolonged Valsalva maneuvers (10, 11).

Macklin and Macklin (12), in 1944, suggested that the causative mechanism of spontaneous pneumomediastinum was development of a pressure gradient between the marginal alveoli and the surrounding blood vessels, with subsequent rupture of the alveoli walls. Any condition resulting in hyperinflation of the alveoli without concomitant dilatation of the blood vessels would create such a gradient.

Once the alveolar wall has ruptured, air dissects along the sheaths of the pulmonary vessels to the mediastinum. It may then follow the fascial planes along the trachea and esophagus into the anterior mediastinum and the subcutaneous tissue of the neck and chest. With further increases in pressure, the pleura may rupture causing pneumothorax, usually on the left. Air may also track along the great vessels into the retroperitoneum (2, 12).

The most common initial symptom of spontaneous pneumomediastinum, seen in 80%–90% of cases, is acute, sharp, substernal pain, often radiating to the neck and back (13). Pleuritic chest pain and dyspnea are also common. Marked respiratory distress is rare. Other manifestations include dysphagia and throat and neck pain (2, 6, 8, 13). Criteria for diagnosis include subcutaneous emphysema without apparent cause, air in the mediastinum on x ray, the presence of Hamman sign—a crunching sound heard along the sternal border synchronous with systole—and decreased cardiac dullness (5, 14). All are rarely present in the same patient, and Munsell (8) maintains that the first two findings are sufficient for diagnosis.

Spontaneous pneumomediastinum is usually a benign condition, requiring only supportive care. Antibiotics are usually not indicated, and resolution usually occurs within several days. The presence of a large amount of air, however, may result in greatly increased pressure and grave cardiac and respiratory disturbances, including decreased venous return and cardiac output, hypotension, and impaired respiration (2, 3).

Tension pneumomediastinum must be treated immediately. Ninety-five percent oxygen is employed to relieve dyspnea and facilitate the reabsorption of nitrogen. Failure to improve rapidly or evidence of cardiac decompensation without pneumopericardium requires surgical intervention. A cervical mediastinotomy via a suprasternal incision is the procedure of choice. The incision must open the pretrachial fascia to decompress the mediastinum effectively. Cardiac decompensation with pneumopericardium is more likely, and pericardiocentesis should be performed if signs of tamponade develop. Asymptomatic pneumopericardium requires no therapy (4).

Summary. Spontaneous pneumomediastinum is rarely seen by the surgeon. It is almost always a benign, self-limited condition resulting from increased intraalveolar tension gradients, with alveolar rupture, usually secondary to acute, intermittent airway obstruction. It must be considered in the differential diagnosis of any patient presenting with free air in the mediastinum who has a history of emesis, heavy exertion, inhaling alkaloidal cocaine, or any other condition resulting in prolonged Valsalva maneuvers. It is critical to differentiate this condition from spontaneous or traumatic perforation of the esophagus, which is potentially fatal and requires immediate and aggressive surgical treatment. The diagnosis of esophageal perforation is established by esophagogram (6, 7).

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Anal Melanoma: Case Report and Review

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In 1857, Moore (1) reported the first case of anal melanoma. Since that time, approximately 457 cases have been reported in the medical literature (2). Malignant melanoma makes up approximately 1%–2% of all cancers in the United States (3–5). Less than 1% of all melanomas are found in the anorectal region (5, 6). Primary malignant melanoma comprises 0.25%–1.25% of all tumors of the anus (5, 6). Thus, primary anal melanoma is an uncommon malignant lesion.

Treatment of this lesion has been uniformly poor. The average survival time is reported to be 25 months; approximately 6% of patients survive five or more years (2, 7). Survival is related more to the stage of the disease and the thickness of the tumor than to the mode of treatment (2, 5, 7–10). However, reports of unexpected successes (11) point up the importance of continued aggressive management of these patients.

Case Report

In February 1984 a 64-year-old white woman consulted us for a single episode of bright-red blood passed by rectum. Rectal examination revealed an oval-shaped, 2-cm, rock-hard mass, rounded and immovable, attached to the mucosal surface on the posterior aspect of the anal canal. The stool did not contain blood. No inguinal lymphadenopathy was present. Examination by colonoscopy was negative except for a dark, polypoid, 2-cm mass at the posterior pectinate line. Biopsy revealed melanoma. No other lesions were noted on chest x ray, intravenous pyelograph, or computerized scan of the abdomen. Laboratory values, including liver function tests, were normal.

Abdominoperineal resection was performed in April 1984. No liver metastasis or other intraperitoneal disease was noted. Iliac nodes were neither enlarged nor pigmented.

The pathologic finding was a polypoid melanoma at the anorectal junction (Fig. 1). The tumor was superficially ulcerated and invaded the superficial layers of smooth muscle. Adjacent squamous mucosa exhibited in situ melanoma and atypical melanocytic hyperplasia. The tumor did not extend to resection margins or regional lymph nodes. The thickness of the tumor determined by the method of Wanebo and co-workers (2, 7, 8), was 8 mm.

The patient is currently alive and receiving adjuvant chemotherapy consisting of Velban.

Discussion

The current treatment of anal melanoma is surgical excision. The two surgical procedures that have been advocated are wide local excision and abdominoperineal resection, each accompanied at times by lymph node dissection. In the literature from 1980 to June 1984, we found no evidence to support one surgical procedure over another (8, 11–13), and no statistical difference in survival between wide local excision and abdominoperineal resection.

The importance of regional lymph node involvement has also been controversial. Wanebo et al in 1981 reported a statistically significant difference between the median survival of lymph-node-negative and lymph-node-positive patients, but unfortunately, no significant difference in five-year survival (8, 13). In addition, Stearns et al suggested in 1980 that the addition of prophylactic groin or pelvic dissection did not alter the prognosis (12). Others have supported this view, and today, prophylactic lymph node dissection has been abandoned by most surgeons (2, 4, 6, 11). If lymph nodes are clinically palpable

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or are retrieved at operation, lymph node dissection has been advocated because it may improve local and regional control of the disease (2, 10, 14).

Other modes of treatment include radiotherapy, chemotherapy, and immunotherapy. In the 1930s local treatment with radiotherapy was used. Today, radiotherapy does not appear to have a role in treatment of the primary lesion (12); it has not increased survival significantly (5, 6, 11, 14). Several authors have suggested radiotherapy for palliation (2, 8, 12).

Chemotherapy may be useful (4, 8, 10). Agents such as cyclophosphamide, vincristine, vinblastine, hydroxyurea, Adriamycin, and more recently decarbazine (DTIC), carmustine (BCNU), lomustine (CCNU), and semustine (MeCCNU) have been studied (4, 10, 12). At present, DTIC is the most effective single drug for disseminated melanoma, with a reported response rate of 20% (10). To date, no significant increase in survival has been seen with chemotherapeutic treatment for anal melanoma (5, 6, 10, 12, 14, 15).

Immunotherapy has also been explored. Both local regression of tumor (12) and a prolonged symptom-free interval (4) with the use of BCG vaccine have been reported in the literature. The overall results, however, have not been dramatic (5, 14, 15), and it is still too early to judge the significance of immunotherapy (6).

Despite this multiplicity of methods of treatment, the prognosis for these patients has not altered.

The stage of the disease and the thickness of the tumor appear to be the most important prognostic determinants. Stage I disease is localized disease with nonpalpable lymph nodes; stage II disease is localized disease with palpable lymph nodes; and stage III disease is advanced disease with a large primary or distant metastases (8). Staging by the thickness of the tumor has been useful. Wanebo et al., using the technique of Breslow (16), which measured the maximal thickness of cutaneous melanomas in terms of the depth of invasion, devised a microstaging approach for anal melanomas that categorized the depth of invasion in millimeters, into levels, beginning from the surface of the epithelium and extending down to the point of deepest penetration. Level I signifies that the tumor is confined to the epithelium; level II, that the tumor has penetrated 1 mm or less; level III, penetration to a depth between 1 and 2 mm; and levels IV and V, penetration more than 2 mm into the muscles and adipose tissue (2, 7). Clark's classification (17), a staging method that assesses tumor thickness for cutaneous melanomas, but in terms of five descriptive anatomic levels based on the anatomy of the skin, is not considered appropriate because of the absence of the classic dermatologic layers of the skin in the anorectal region (18)—that is, there is no papillary dermis in the anal canal, where these tumors are usually located (2, 12).

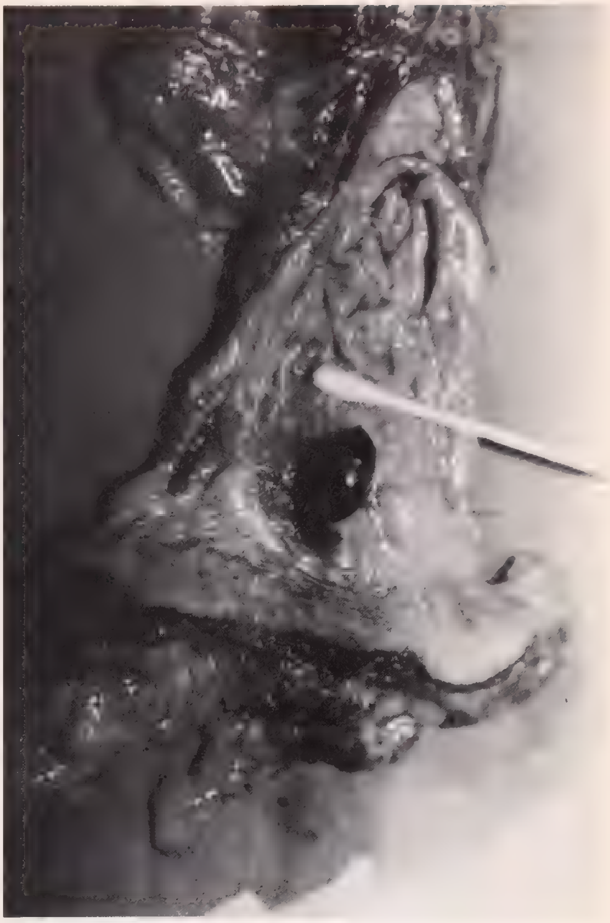

Fig. 1. Surgical specimen—applicator stick adjacent to melanoma at dentate line of anorectum.

As the stage of the disease advances, the prognosis becomes progressively worse (8). Perhaps more significant than the stage of the disease, though, is the finding that those patients who had tumors less than 2 mm thick had extremely good prognoses (2, 7–9, 12). Whereas overall survival of anal melanoma is approximately 25 months (2), patients with lesions less than 2 mm thick have survived 10 or more years and have remained clinically free of disease (11). The five-year survival rate for patients with tumors less than 2 mm thick is 100% (8, 12). In contrast those patients who had tumors 2 mm thick or larger had extremely poor prognoses; their five-year survival rate is close to zero (2, 8, 12), and only one patient survived beyond five years (11).

That survival can be long-term after both wide local excision and abdominoperineal resection (11, 12) adds further support to the contention that survival after these two surgical procedures is not statistically different. It may, however, be impossible to perform a wide local excision without causing significant sphincter damage, rendering the patient incontinent; therefore, abdominoperineal resection may be the procedure of choice. In the patient described here, the tumor invaded the sphincters; thus abdominoperineal resection was indicated. An additional advantage of abdominoperineal resection over wide local excision, for patients with microscopically small anal melanomas, is that only with such a resection can clinically undetectable regional lymph node metastases be excised (13). Although this may not change the overall prognosis, it may improve local and regional control of the disease (2, 10, 14) as well as decrease the risk of recurrence (2).

An additional point arises from Freedman's report (11) of two patients whose survival was prolonged. One had stage II, level V (13 mm) disease, the other had possible stage III (level not available) disease. Both were free of disease after 10 years. Freedman's report serves to emphasize the fact that unexpected results can occur with aggressive treatment.

Summary

Clinical staging and microstaging of anal melanoma is crucial in determining prognosis. Early diagnosis is currently the only effective way of improving the survival rate. Although abdominoperineal resection offers no proven advantage over wide local excision in altering survival, adequate resection of the lesion may preclude preserving sphincter function. In such cases, abdominoperineal resection may be the only alternative. Adjuvant therapies have yet to have an impact on the survival rate. Currently, their usefulness appears limited to palliation.

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Ileal Endometriosis Causing Acute Small-Bowel Obstruction

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Abstract

A case of ileal endometriosis causing small-bowel obstruction is reported. This case illustrates the difficulty in making a preoperative diagnosis short of surgical exploration. The pathogenesis of small-bowel obstruction by enteric endometriosis and the pertinent literature are discussed.

Enteric endometriosis is a rare cause of small-bowel obstruction. Although gastrointestinal involvement by endometriosis is common, the majority of patients are asymptomatic. Symptomatic small-bowel involvement is very unusual. We report a case of subacute small-bowel obstruction by ileal endometriosis.

Case Report

A 34-year-old black woman was admitted to the University of Michigan Hospital in March 1984 with abdominal pain, nausea, and vomiting of two weeks' duration. She had a two-year history of chronic intermittent abdominal pain associated with nausea and vomiting. The first episode of this pain occurred six weeks following cesarean section two years previously. Her most recent episode of pain occurred in January 1984, two months prior to the present admission, and lasted four or five days.

The woman continued to be mildly symptomatic between episodes for approximately six months. She had lost 30 lb of weight over the previous 18 months. She did complain that her pain seemed to worsen at the time of her menstruation, but the association was not clear-cut. During the previous two years she had an extensive evaluation at another institution, including upper-gastrointestinal series, small-bowel follow-through, barium enema, intravenous pyelogram, abdominal and pelvic ultrasound, and upper and lower endoscopy. All studies were normal except for endoscopic evidence of "duodenitis." She had been treated with Ranitidine with minimal improvement, but her symptoms recurred. Her history was significant for hypertension for the past 17 years controlled on medication. She is gravida 2, para 1 and was known to have uterine fibroids. She had one spontaneous abortion. Her cesarean section was performed for preeclampsia. She had no history of peptic ulcer disease.

Physical examination revealed an alert woman in moderate abdominal pain with a temperature of 100.8°F, pulse rate 100/min, and blood pressure of 174/118. Bowel sounds were hyperactive. Tenderness was noted in the midabdomen and left lower quadrant. There was no rebound tenderness or peritoneal signs. No masses were palpable in the abdomen. Vaginal examination revealed that she was menstruating at the time. The adnexa were nontender, and the rectal examination was normal.

Laboratory studies revealed a mild hypokalemic alkalosis with no other significant abnormalities. Abdominal radiographs revealed an acute small-bowel obstruction pattern with progression over two successive days (Fig. 1). Pelvic

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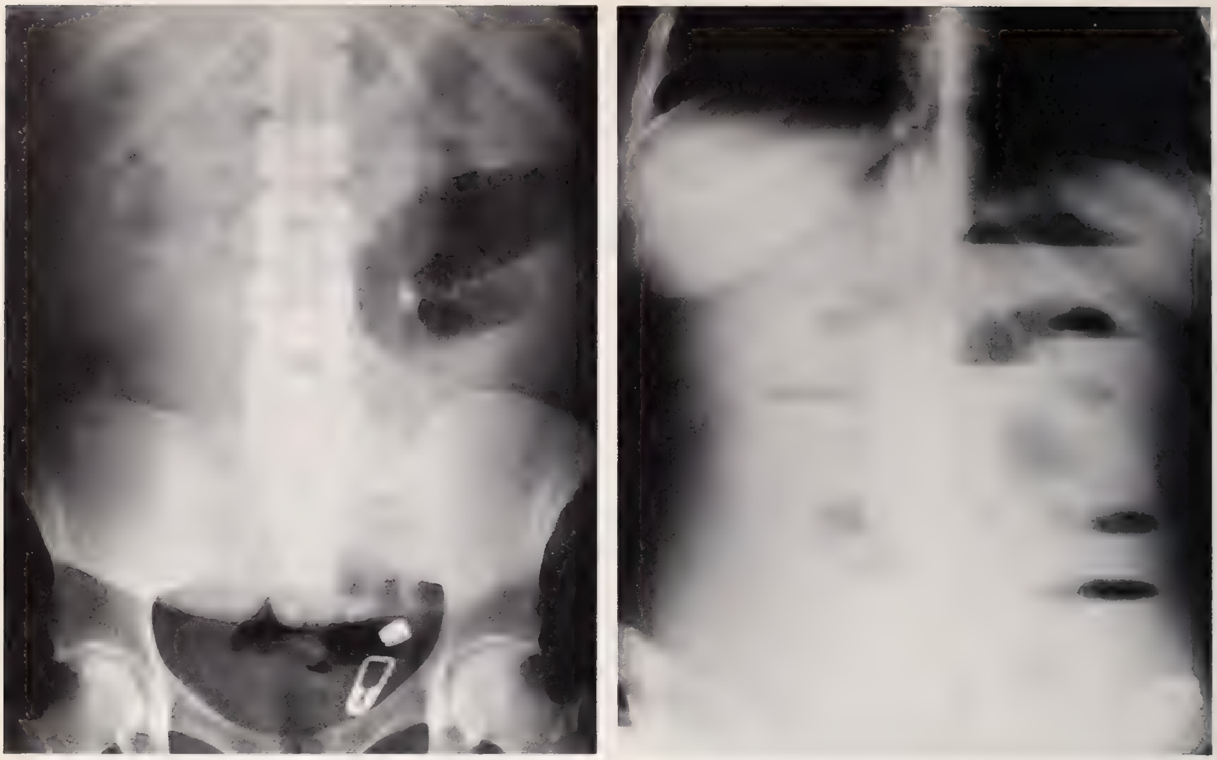

Fig. 1. Supine (**left**) and upright (**right**) views of abdomen reveal distended loops of small bowel with multiple air-fluid levels consistent with acute small-bowel obstruction.

and abdominal ultrasound revealed distended loops of bowel and no distinct mass lesion.

The patient was hydrated and underwent an exploratory laparotomy on the third day of her admission. A complete small-bowel obstruction was found. The point of obstruction was located in the distal ileum 10 cm proximal to the ileocecal valve. A tight adhesive band was present which could not be reduced. Resection of a 15-cm segment of ileum with primary ileoileostomy and appendectomy was performed. The final histopathologic diagnosis was endometriosis of the ileum (Fig. 2). The appendix was normal.

The postoperative course was unremarkable. The patient was referred to the gynecologist for possible hormonal therapy for further management. A one-year follow-up revealed no recurrence of symptoms.

Discussion

Endometriosis is characterized by the presence of ectopic endometrial tissue in an extramyometrial site. It affects 10%–15% of women during their reproductive years and is commonly associated with uterine leiomyomas, ovarian follicular cysts, and endometrial polyps (1). Kistner found that endometriosis may be present in 18% of women undergoing laparotomy for gynecologic reasons (2). The most common sites of endometriosis, in decreasing order, are ovaries, the pouch of Douglas, uterine ligaments, pelvic peritoneum, rectovaginal septum, uterine cervix, and inguinal hernial sacs. Of 1,000 consecutive celiotomies in gynecologic practice, Williams and Pratt found pelvic endometriosis in 485 patients with enteric involvement in 181. Only one of these patients was symptomatic and operated on for small-bowel obstruction (3). In a review of 7,177 cases of endometriosis, McAfee and Greer (4) found gastrointestinal tract lesions in 12% of patients; 85% of these lesions were in the rectosigmoid, 7% in the small bowel, 3.6% in the cecum, and 3% in the appendix. In a Mayo Clinic series of 7,200 cases of endometriosis, involvement of small intestine was present in 36 patients (incidence: 0.5%). The majority of those cases were found incidentally at surgery, and only 11 patients presented with symptomatic small-bowel obstruction (5).

Ileal endometriosis commonly involves the terminal portion of the ileum. Melody (6), in a review of 36 cases of ileal endometriosis, found that in 35 patients the involved segment was the last 10 cm of terminal ileum. Involvement of the ileum proximal to Meckel's diverticulum is rare (7), but two cases of endometriosis involving

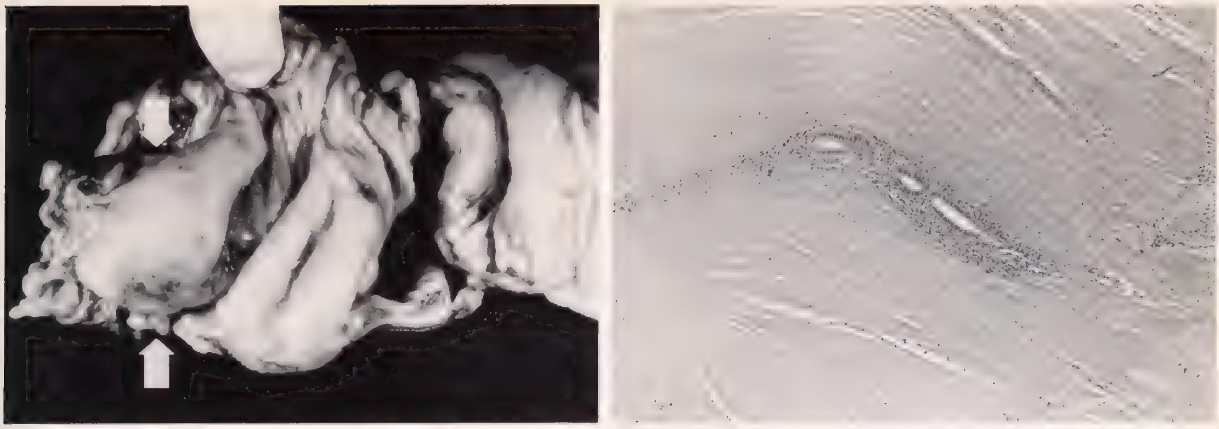

Fig. 2. **Left** Gross specimen of resected ileum shows thickened mucosa with 1.5-cm nodular lesion (arrows); overlying mucosa is flattened with a 0.3 × 0.5 cm ulcer and hemorrhagic changes. Circumference of bowel narrows from 7 cm to 3 cm at site of nodular lesion. Bowel wall is thickened and serosa is roughened. **Right** Photomicrograph of small-bowel wall from area of nodular thickening shows endometrial glands and stroma interdigitating with smooth muscle (hematoxylin and eosin ×75, original magnification).

Meckel's diverticulum have been reported (8, 9). Enteric endometriosis is usually subserosal with less frequent involvement of the muscularis propria and mucosa (10).

The clinical features of symptomatic small-bowel endometriosis are nonspecific. Acute as well as chronic abdominal symptoms with nonspecific features may pose diagnostic and therapeutic challenges. The presenting symptoms may be indistinguishable from those of acute appendicitis, intestinal ischemia, or acute Crohn's disease. Fever, leukocytosis, and even peritoneal signs may develop with enteric endometriosis and suggest an acute surgical abdominal emergency. Acute, chronic, or intermittent intestinal obstruction may develop; acute small-bowel obstruction as a feature of endometriosis is rare, and only 30 cases have been recorded (4, 7, 11–19). Enteric endometriosis frequently, but not always, manifests as a temporal relationship between the onset of symptoms and menstruation. Pelvic examination usually reveals pelvic endometriosis, although the small intestine may be the only site of involvement.

Endometriosis of the small bowel has also been described in postmenopausal women who may or may not be taking medication containing estrogen (20). There is no clear-cut relationship between the extent of the disease and the severity of the symptoms. Endometriotic foci may be insensitive to ovarian hormones, but generally they respond to the cyclical secretion of ovarian hormones similar to normally situated endometrium. The recurrent proliferation, engorgement, swelling, and "menstruation" from the foci of endometriosis result in local extravasation of blood. The release of this intensely fibrogenic iron promotes fibrosis and adhesions. These two factors, recurrent swelling and formation of dense fibrous adhesions, are responsible for most of the symptomatology of endometriosis.

The pathogenesis of ileal obstruction is probably multifactorial. A localized endometrial mass (endometrioma) may encroach upon the lumen and, aided by the presence of adhesive bands, may cause acute small-bowel obstruction (4, 7). More commonly diffuse endometriosis is seen with mucosal thickening and consequent narrowing of the lumen with true stricture formation. Intestinal volvulus and intussusception have also been reported with distal small-bowel endometriosis (7, 11). Less than 50% of patients with small-bowel endometriosis have other pelvic organ involvement (6, 13, 21–26).

The preoperative diagnosis of endometriosis as an underlying cause of small-bowel obstruction is difficult. Despite an occasional temporal relationship of abdominal pain to menstruation, the clinical features are nonspecific, and most diagnostic tests are unrewarding in differentiating enteric endometriosis from other inflammatory or neoplastic disorders that may obstruct or infiltrate the bowel. The diagnosis is usually made at surgery and is readily confirmed by histopathologic identification of typical endometrial glands, stroma, and menstrual changes.

Endometriosis is a progressive disease, and recurrence of symptoms is common despite therapy. In the short follow-up of one year in our patient there was no recurrence of symptoms.

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Instructions for Authors

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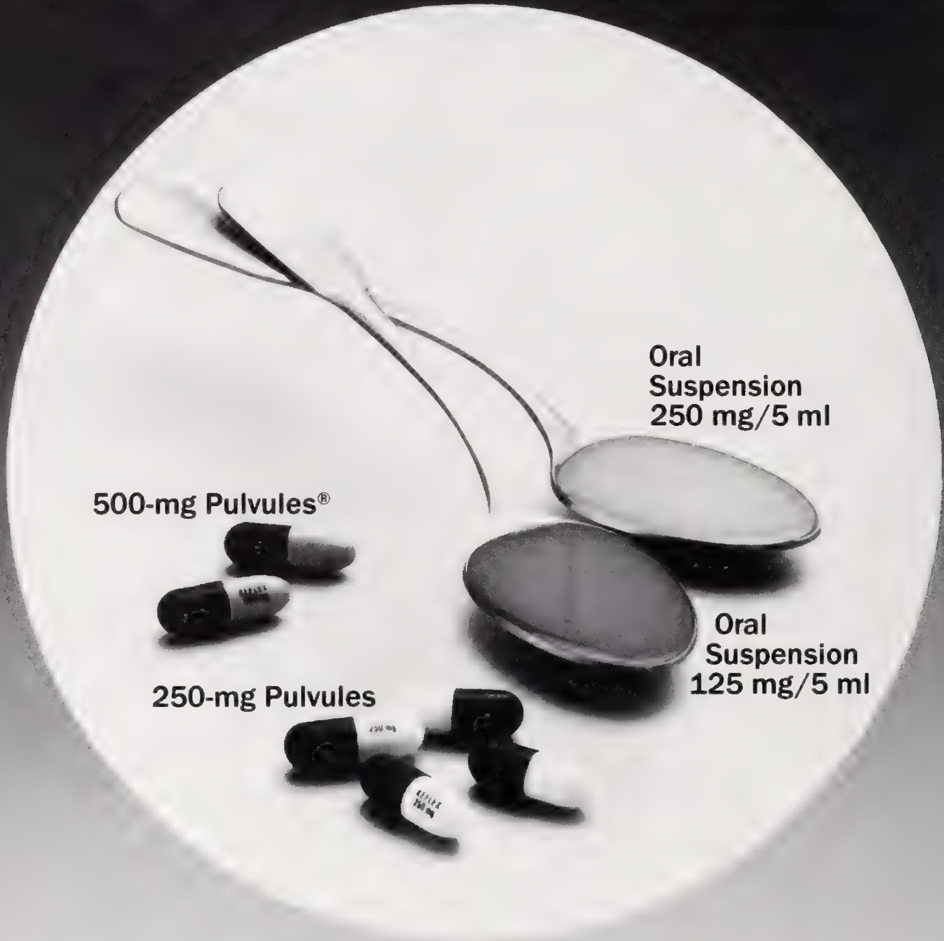
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Near-Total Splenectomy for Massive Splenomegaly due to Gaucher Disease: A New Surgical Approach

LEON MORGENSTERN, M.D., FACS, EDWARD H. PHILLIPS, M.D., FACS, DAVID FERMELIA, M.D., AND IRWIN M. WEINSTEIN, M.D., FACP

Abstract

The standard surgical treatment for massive splenomegaly and hypersplenism due to Gaucher disease has been total splenectomy. However, because total splenectomy may lead to an acceleration of bone destruction and have other adverse consequences, near-total splenectomy was considered as an alternative operation to total splenectomy in this disorder. With this in mind, two patients with known Gaucher disease, massive splenomegaly, and life-threatening hypersplenism were subjected to near-total splenectomy, all but a normal-sized splenic remnant being resected. Both patients had an uneventful postoperative course and on follow-up, at 20 months for the first patient and 12 months for the second, the initial therapeutic objectives have been achieved.

Splenomegaly is a constant feature of advanced type I Gaucher disease, the sphingolipidosis caused by deficiency of the enzyme glycosyl ceramide—B glucosidase (1, 2). Progressive splenomegaly, often to massive proportions, may eventually present two cogent indications for splenectomy: the mechanical phenomena associated with the large splenic mass alone, or hypersplenism with pancytopenia.

Splenectomy is usually deferred as long as possible. When indicated, the standard surgical procedure is total splenectomy. The primary indication for splenectomy from the hematologic standpoint is the severity of the thrombopenia and leukopenia, which are more frequently the consequence of hypersplenism than marrow involvement with Gaucher cells. However, the adverse effects of total splenectomy in Gaucher disease, namely increased susceptibility to infection, accelerated hepatomegaly, and, most importantly, accelerated bone destruction, have led to a reappraisal of the standard surgical approach. Near-total splenectomy has been proposed as an alternative operation to avoid the untoward consequences of total splenectomy. This report describes near-total splenectomy in two patients with adult type I Gaucher disease and reviews the current world experience with this novel approach to the surgical treatment of this disorder.

Case Reports

Case 1. A 41-year-old Jewish woman with long-standing Gaucher disease developed massive splenomegaly and worsening hypersplenism. Multiple ecchymoses of her lower extremities were consequent to thrombocytopenia as low as 24,000 cu/mm. The only other clinical manifestations of her disease were abdominal discomfort due to the massive splenic enlargement and right hip pain. A sister also has Gaucher disease.

The most prominent physical finding was an enlarged spleen extending into the pelvis and across the midline. The liver was not enlarged to palpation.

Laboratory examination revealed a hemato-

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crit of 30, and a leukocyte count of 1,900 cu/mm. Bone marrow studies showed extensive infiltration with Gaucher cells. All normal elements were reduced in numbers. Radiologic examination of the pelvis and hips showed osteonecrosis of the right femoral head and osteoporosis of the pelvis.

At operation the surgeon (EHP) found the spleen massively enlarged, with evidence of previous upper pole infarction (Fig. 1). The liver showed moderate to marked enlargement. A near-total splenectomy was performed, leaving an inferior polar splenic remnant weighing an estimated 150 gm (Fig. 1). The operative blood loss was measured at 400 cc. One unit of donor-directed packed red blood cells was given and one pack of fresh platelets, obtained by donor-directed platelet pheresis, was also administered.

The excised spleen weighed 4,230 grams. Microscopic examination showed marked infiltration with Gaucher cells, which showed strong cytoplasmic staining for tartrate-resistant acid phosphatase.

The postoperative course was uncomplicated. The platelet count rose to 603,000 cu/mm prior to her discharge on the seventh postoperative day.

At the time of this report, 20 months after operation, the patient is asymptomatic except for mild bruising. There is no palpable hepatomegaly and the splenic remnant cannot be felt. The platelet count is 118,000 cu/mm, the hemoglobin is 11.9 g/dL, the hematocrit 36.7%, and the leukocyte count 6,000 cu/mm.

A postoperative radionuclide scan (Fig. 2) shows a normal-sized splenic remnant.

Case 2. A 44-year-old Jewish woman with known Gaucher disease of 17 years' duration was hospitalized for symptomatic thrombocytopenia secondary to hypersplenism. She had noted progressive easy bruisability, gingival bleeding, epistaxis, and excessive bleeding after minor trauma over a three-year period. Her spleen was known to have been enlarging but caused her no physical discomfort. The only musculoskeletal symptoms were occasional mild arthralgias.

On admission, physical examination showed scattered ecchymoses indicative of easy bruisability. The spleen filled the left flank, extending downward to the left iliac crest and medially to the midline. The liver was palpable two fingerbreadths below the costal margin.

Laboratory data included a hematocrit of 32, a leukocyte count of 3,200 cu/mm, and a platelet count of 25,000 cu/mm. Liver function test results were mildly elevated. Bone marrow examination showed extensive involvement with Gaucher cells, but adequate numbers of megakaryocytes. Bone x-rays showed slight widening of the marrow cavity in the distal femoral shafts.

At operation (EHP), the most significant finding was massive splenomegaly (Fig. 3). A near-total splenectomy was performed using the same technique as in Case 1, preserving a lower pole remnant estimated to weigh between 150 and 180 grams (Fig. 3). Intraoperatively the patient required one fresh platelet transfusion (obtained by platelet pheresis) and four units of packed red cells.

The resected spleen weighed 1,390 grams. Microscopic examination showed marked infiltration with Gaucher cells and scattered foci of extramedullary hematopoiesis.

Postoperatively the patient had a mild febrile course, the source of which was never elucidated with certainty; it was thought to be due to possible localized infarction of the splenic remnant. However, a postoperative radionuclide scan showed a splenic remnant the size of a normal spleen (Fig. 4).

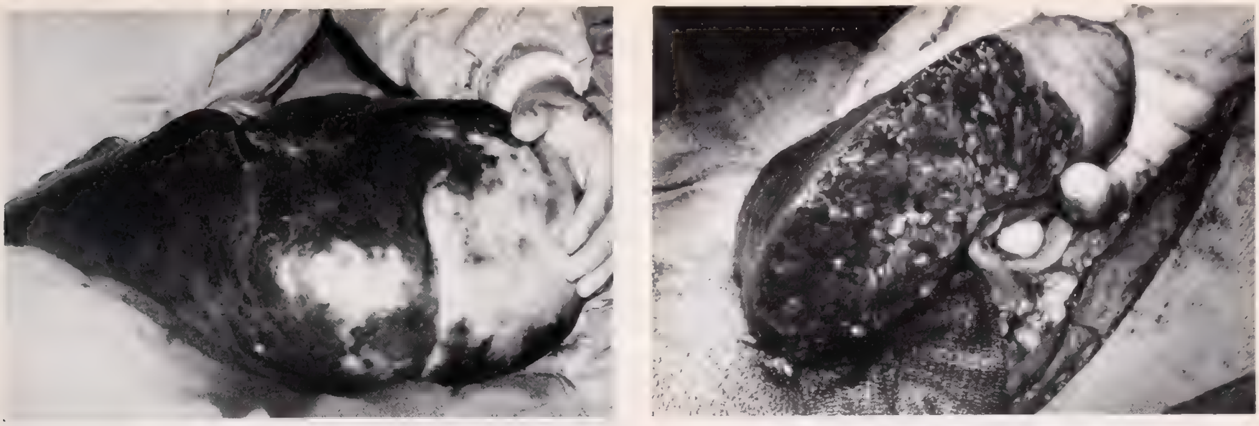
The postoperative platelet count rose to 447,000 cu/mm by the time of discharge on the sixteenth postoperative day.

At the time of the present report, eight months after operation, the patient is asymptomatic. Her platelet count has stabilized at 50,000 cu/mm but there is no evidence of a bleeding tendency. On physical examination the splenic remnant is not palpable. The hepatomegaly is unchanged. There are no musculoskeletal symptoms.

Discussion

Since its description by Gaucher in 1882, the disease which bears his name has defied effective long-term therapy. Although splenectomy had been performed for splenic manifestations of the disease as early as 1896 (3), it is only recently that sizable numbers of cases have been reported. Regrettably, there are few follow-up reports which enable us to assess the long-term results of splenectomy for Gaucher disease. Notable exceptions are the reports of Marks et al (4), Aufses and Salky (5), and Shiloni et al (6). Gleaned from these reports is the general impression that relief of mechanical symptoms is constant, hematological problems (thrombocytopenia, anemia, leukopenia) are usually improved, and the destructive process in bone may or may not accelerate.

Partial or near-total splenectomy, as per-



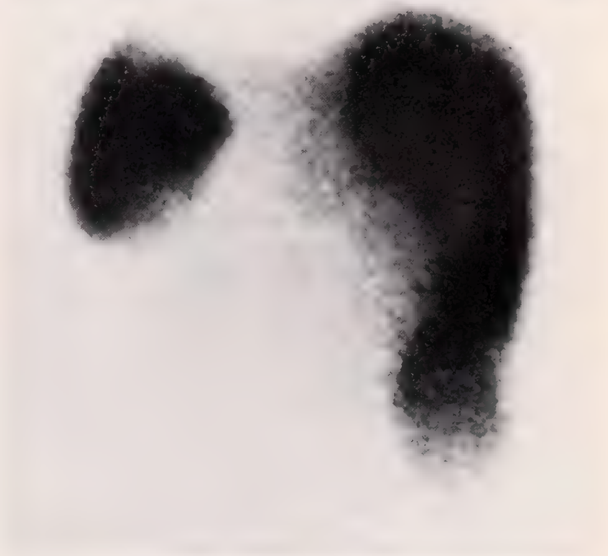


Fig. 1. Patient no. 1. **Left** Spleen after mobilization, showing massive splenomegaly and evidence of previous upper pole infarction. **Right** Splenic remnant, lower pole, after resection of major portion of spleen.
Fig. 2. Patient no. 1. Postoperative Technetium 99 scan, showing splenic remnant.

formed in the two patients we report, has become well-established as a feasible surgical technique in the past two decades. Its principal application has been in the field of traumatic injuries to the spleen, but it has also been described for splenic cysts (7), schistosomiasis (8), and myelofibrosis (9).

The first reference to partial splenectomy for Gaucher disease was made by Beutler in 1977 (10), citing the case performed by Yonemoto. Govrin-Yehudain and Bar-Maor, in 1980, reported a successful partial splenectomy in a young adult with type I Gaucher disease (11) and in 1985 described three additional partial splenectomies in children (12). Marcel Campos Christo, the true pioneer in the technique of subtotal splenectomy, in a written communication to one author (LM) (13), has cited three successful cases of partial splenectomy for Gaucher disease. Another has been verbally described to the same author, by Aufses in 1984 (14). The two cases in this report bring the total of known patients treated by partial splenectomy to eleven, although there are probably others which have been unreported. Only the first case, that performed by Yonemoto, was done by an unconventional technique as diagrammed by Beutler (10). The remaining operations were done with the technique described by Morgenstern et al (15), and were accomplished without undue difficulty. In the published cases, the lower pole seems to have been the site chosen for the splenic remnant for technical reasons. Although all patients recovered successfully from the surgical procedures, no long-term follow-up describing recurrence or progression of the hematologic or osseous abnormalities has as yet been reported.

Partial splenic embolization has been described by Thanopoulos and Frimas (16) in a 4-year-old child with the juvenile form of Gaucher disease. There was a “significant reduction” in splenic parenchyma with improvement in the hypersplenic manifestations (leukopenia, thrombocytopenia). Twelve months following the embolization, the leukocyte and thrombocyte counts remained within the acceptable range (5,000 cu/mm and 100,000 cu/mm respectively).

Since the rationale of near-total splenectomy or partial splenic ablation is based on the premises of preservation of immunologic competence, the correction of hematologic deficits, and the prevention of hepatic enlargement and bone destruction, the following questions still remain unanswered.

Unanswered Question No. 1: Sepsis. First, is the rate of infection, or overwhelming postsplenec-

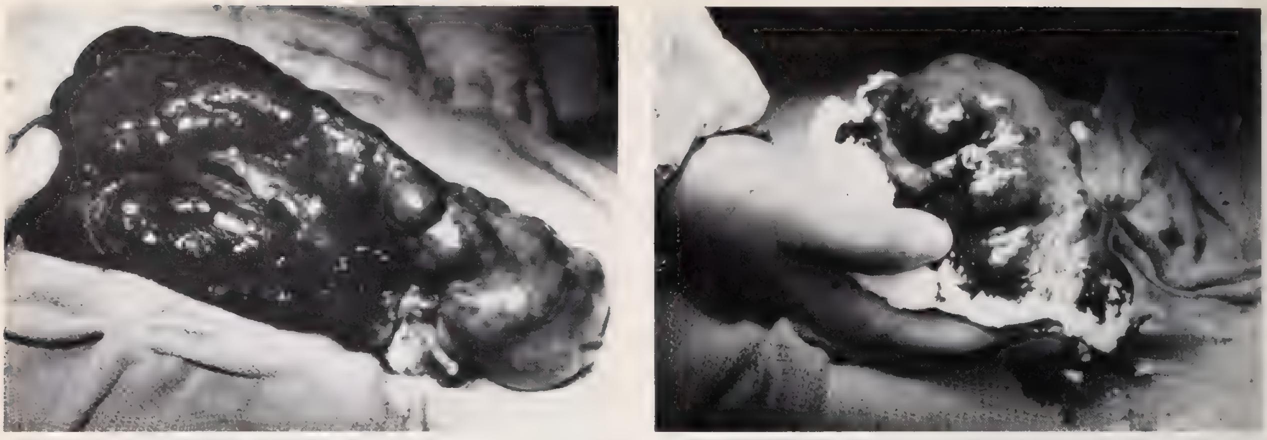


Fig. 3. Patient no. 2. **Left** Spleen following mobilization and after ligation of all vessels but those to inferior pole. Note clear demarcation of vascularized portion. **Right** Splenic remnant, lower pole. Avitene has been applied to raw surface for topical hemostasis.

tomy sepsis, a documented phenomenon in patients who have been splenectomized for Gaucher disease? The answer is not known, since there have been no cases of overwhelming postsplenectomy sepsis reported in such patients. However, it is known that splenectomized patients, especially those splenectomized for hematologic disorders, are prone to overwhelming postsplenectomy sepsis (17), although in low incidence, and the occurrence of such a phenomenon in patients splenectomized for Gaucher disease might be expected eventually. The retention of a viable splenic remnant with good function, as shown by radionuclide imaging, would seem to afford that measure of protection needed to avoid the consequences of decreased immunologic competence.

Unanswered Question No. 2: Sequelae. Second, what are the long-term hematologic sequelae of near-total splenectomy as contrasted with total splenectomy? These too are as yet unknown. With total splenectomy, the hematologic improvement seems to be permanent and satisfactory. Whether the same will hold true in patients with a splenic remnant remains to be seen. It is to be expected that the splenic remnant will enlarge as it becomes a locus for deposition for glucocerebroside. Should it enlarge to massive proportions, the same situation would again exist which favored the occurrence of the hypersplenism originally. In the authors' opinion, based on previous experience with splenic remnants, accessory spleens, and splenic implants in humans, the prediction is that the spleen will reach a finite size based on its circulatory sufficiency and will then cease enlarging. In our first

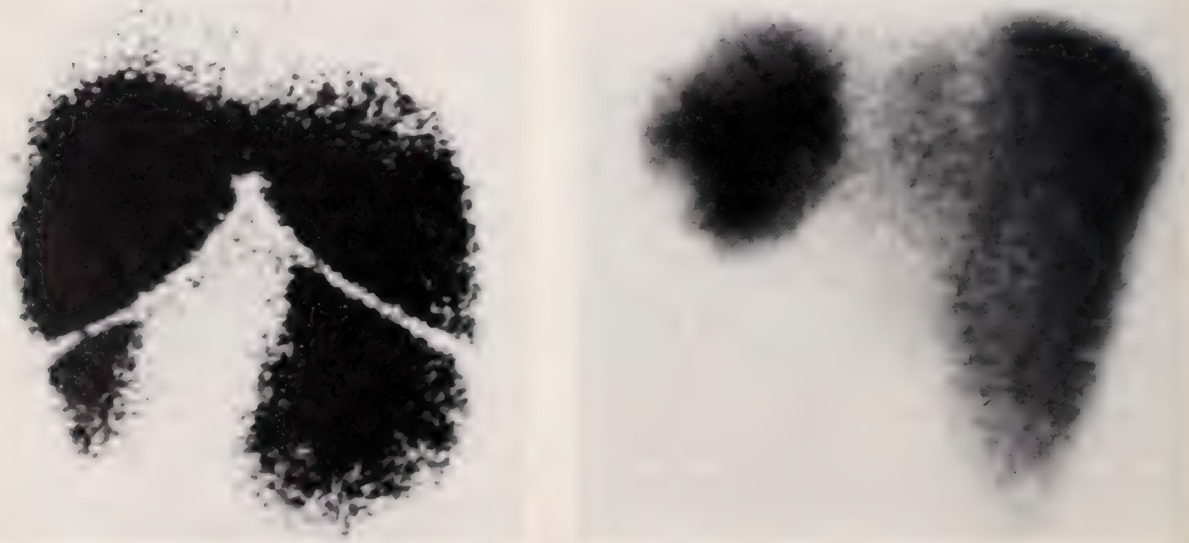


Fig. 4. Patient no. 2, Technetium 99 scan. **Left** Preoperative (anterior view). **Right** Postoperative (posterior view).

patient, 20 months after splenectomy, the spleen is not palpable and is not appreciably enlarged on radionuclide scan. Nevertheless, it must be admitted that this question still remains unanswered and is a topic for future investigation and reporting.

Unanswered Question No. 3: Bone Status and Liver Size. Third, will near-total splenectomy protect against accelerated bone destruction or hepatic enlargement, which have both been reported to follow total splenectomy in Gaucher disease? The answer is still unknown. Moreover, some dispute exists whether total splenectomy is truly responsible for accelerated bone destruction. Rose et al (18) have reported a case in which bone destruction was accelerated following splenectomy. This also was noted in five of the thirteen patients described by Shiloni et al (6) on long-term follow-up. Goldblatt et al (19), however, doubt the causal relationship between the bone destruction and total splenectomy. It is reasonable to expect increased deposition in liver and bone when the large reservoir for glucocerebroside storage represented by the spleen is removed, but this is an a priori assumption, and the true protective effect of partial or near-total splenectomy against massive hepatomegaly or increased destruction in bone still awaits.

Near-total splenectomy for Gaucher disease thus remains an experimental procedure based on what is known about splenic function in immunologic competence, but based on assumption alone in respect to hematologic sequelae, protection against massive hepatomegaly, and protection against increased bone destruction. Only careful reporting on the patients already operated upon, with careful follow-up data, will supply these answers. It is to be hoped that the primary emphasis will not be on the technical feat of performing an exacting surgical operation, but on the true value of this procedure in protecting against the adverse sequelae of total splenectomy.

Summary

Near-total splenectomy was performed in two adult women with type I Gaucher disease. The primary indication for this procedure in both patients was massive splenomegaly with hypersplenism and marked thrombocytopenia. Splenic remnants approximating a normal-sized spleen were retained. Postoperatively, the thrombocytopenia has improved and there is no evidence of accelerated bone destruction in either patient.

AUTHORS' NOTE: Since submission of this article, an additional series of partial splenectomies for Gaucher disease in children has come to our attention: Rubin M, Yampolski I, Lambrozo R, Zaizov R, Dintsman M. Partial splenectomy in Gaucher's disease. J Pediatr Surg 1986; 21:125–128.

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Detection of Ovulation via Fingertip Electrical Potentials: A Preliminary Report

KENNETH P. STOLLER, M.D., AND PHILIP G. BROOKS, M.D.

Abstract

Electric potentials were measured on the fingertips of three healthy women, each of whom also measured their basal body temperature for two consecutive menstrual cycles. In two patients the mean bilateral potentials showed a clear-cut midcycle rise in the direct-current (DC) potential correlated with the shift in temperature. In the third patient's second cycle a midcycle peak potential preceded the temperature shift by six days; plasma progesterone measurements confirmed ovulation prior to the shift in basal body temperature. The measurement of dermal DC potentials on the fingers of healthy, regularly menstruating women seems to be both sensitive and specific for detecting a bioelectric change in association with ovulation.

Direct-current (DC) potentials have long been described and correlated with a variety of biological functions, from the migratory behavior of the Atlantic eel to the clinically useful bone-growth-stimulation techniques using electric fields. In 1937, Burr et al (1) were able to document the vaginal-cervical bioelectric record of the "instant" of ovulation in a human female, with confirmation at laparotomy. These investigators indicated that it may be possible to obtain bioelectric records of ovulation from such remote points of contact as "the fingertips." Twenty years later, Parsons et al (2) confirmed that vaginal-cervical electropotentials have characteristic cyclic patterns with a midcycle change not found in postmenopausal, irradiated, or surgically castrated women. They also showed that temperature, pH, and psychogenic factors, while allied to differences in potentials, could not produce the repetitive cyclic changes observed during the menstrual cycle in presumed association with periodic ovulation. In 1967, Zwinger and Vrana (3) reached a similar conclusion on observing changes in impedance. In 1985, Albrecht et al (4) used vaginal electrical resistance to confirm ovulation.

This preliminary investigation was undertaken, using contemporary technology, to measure more precisely electric potentials at discrete dermal sources of DC on the fingers, for the purpose of detecting ovulation.

Patients, Materials, and Methods

Three healthy women who had no history of menstrual problems or irregularities measured their basal body temperatures (BBT) and fingertip electrical potentials for two cycles. For one patient (patient 3) with inconclusive BBT curves,

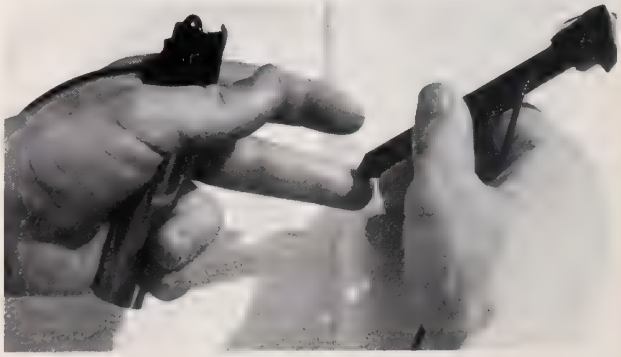

Fig. 1. Probe electrode in right hand measures DC potential of left fingertip. Ground electrode is held in the left hand.

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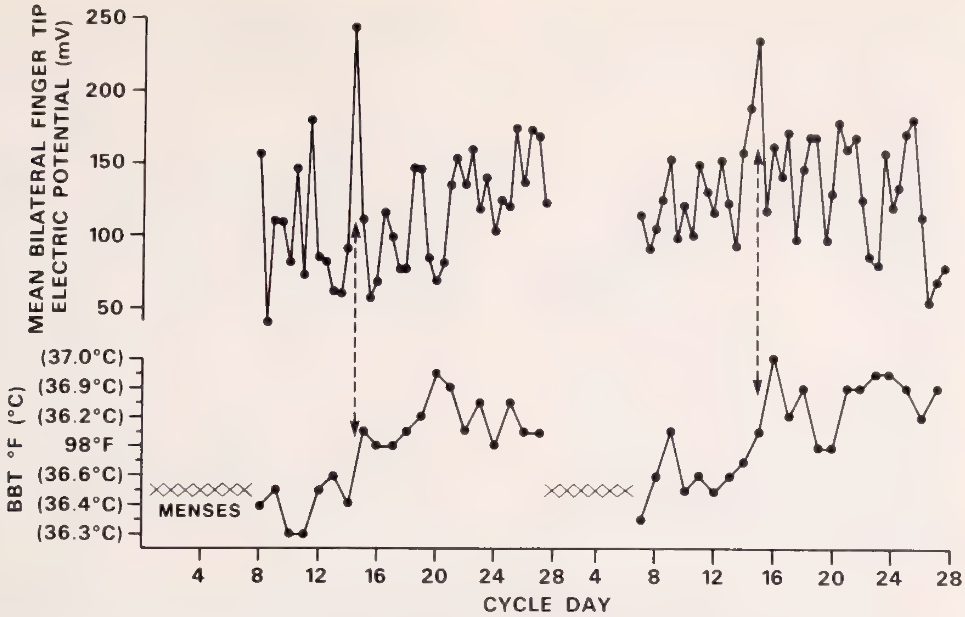

Fig. 2. Patient 1: basal body temperature and DC potentials during two menstrual cycles. Arrows at midcycle peak in potential correlate with shift in basal body temperature.

plasma progesterone was measured on day 20 and 25 of her cycle to confirm ovulation.

Potentials were measured in the 1 μV–700 mV range with a DC digital voltmeter (input impedance, 2 × 10^8 ohms) which utilized automatic gain control and ranging. Noninvasive skin contact was made via two stainless steel electrodes. The ground was a cylindrical element, 101.6 mm × 19.05 mm in diameter. The probe electrode had a ball tip 2.38 mm in diameter and was fully insulated and shielded except for the exposed ball tip. The ground electrode was held by the patient in either the right or the left hand, depending on whether the probe electrode was measuring a point on the left or the right finger (Fig. 1). Clean skin surfaces were unprepared.

A consistent electrode-skin interface is required to obtain reproducible values. This is achieved using a pressure-constant probe, and by gently manipulating the probe tip until the maximum stable value of DC is obtained. The value of the absolute potential at any given DC point-source can alter from one moment to the next under the influence of a number of variables, including physical distress and a change in mood or state of alertness. Therefore, in addition to the measurement of potentials at symmetric points, multiple determinations were made at each point to minimize the possibility of obtaining a false value. Patients were required to be relaxed but not drowsy when taking measurements.

Because of a previous report that the midcycle change in potential representing the ovulatory event might last less than 20 hours (1), patients were instructed to take measurements twice daily, once in the morning after rising and once in the evening at bedtime. Identical locations on each hand were chosen for measurement, because they showed evidence of being optimal low resistance-high conductance areas of the skin as indicated in a previous report (5). This site is located on the radial aspect of the terminal phalanx of the third finger, just proximal to the nail bed.

Results

The mean bilateral electric potentials in patients 1 and 2 show a clear-cut midcycle rise in DC potential correlated with BBT shift (Fig. 2). The midcycle peak in potential seems to correspond exactly to the presumptive time of ovulation indicated by BBT. The significance of the midcycle peak was determined by comparing the mean bilateral midcycle peak potential with the mean of the remaining potentials via the two-sample t test. This analysis indicates that the mean bilateral midcycle peak potential was significantly different from the mean of the remaining potentials for both cycles ($p < 0.0001$). In addition, the difference between measurements of the left and right fingers averaged less than 10 mV for both cycles.

Patient 3 also produced a midcycle rise in po-

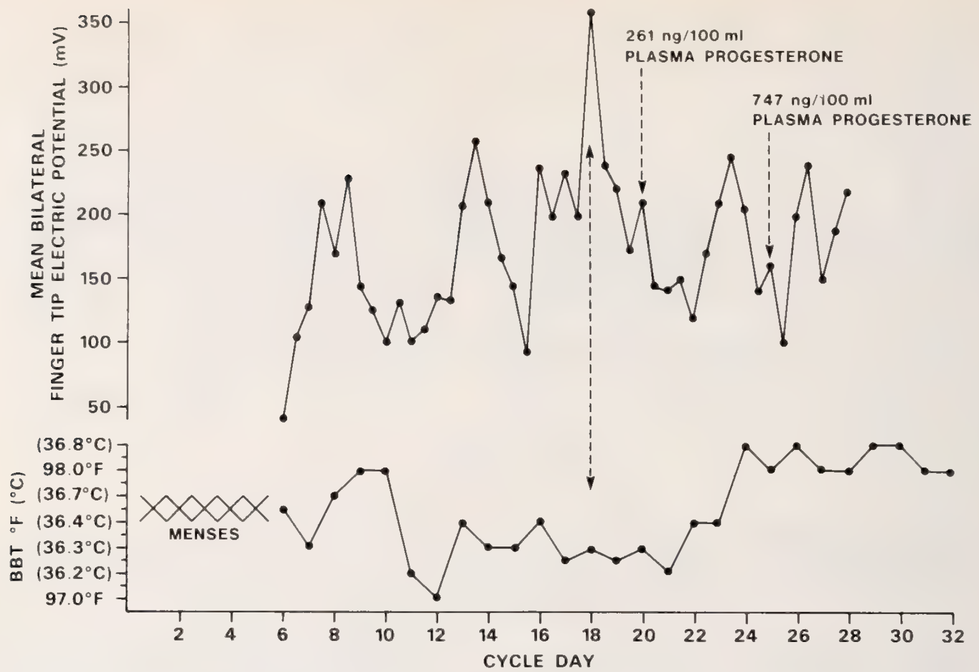

Fig. 3. Patient 3, second menstrual cycle. Midcycle peak in DC potential precedes by six days the shift in basal body temperature. Plasma progesterone measurements confirmed ovulation prior to shift in temperature.

tential for each of her two cycles. Her BBT curve, however, did not shift accordingly. The mean bilateral potentials and the BBT in her second cycle are shown in Fig. 3. Plasma progesterone was measured at two and seven days after the midcycle peak in potential, in order to determine whether this change in potential reflected the beginning of her luteal phase. The respective two- and seven-day values were 261 ng% and 747 ng%, which correspond with luteal phase values that would be predicted two and seven days after ovulation (6). The progesterone values and DC potential measurements both confirm ovulation took place about day 18 of this cycle.

Discussion

The result of this preliminary investigation indicates that the measurement of dermal DC potentials on the fingers of healthy, regularly menstruating women is both sensitive and specific for detecting a bioelectric change in presumed association with ovulation. In addition, each midcycle peak potential occurred in only one morning or evening measurement, but not both. This would seem to indicate that the rise in fingertip DC potential lasts for only 12 to 14 hours, confirming Burr's observation (1).

The only study to definitely confirm ovulation visually and correlate the event to an electrical signal was that of Burr et al (1). Burr and colleagues showed that the surge in electrical potential in the vaginal-cervical area corresponded to the ovulatory event. This surge in potential would also correspond to a nadir in electrical resistance in the same vaginal-cervical area. Based on the work of Albrecht et al (4), vaginal electrical resistance begins to fall up to three days prior to the nadir, and the nadir is not significantly lower than the value on the preceding day. It may be difficult to identify the resistance nadir on the basis of its absolute values. Albrecht et al showed that the resistance nadir corresponded to the LH peak, but were unable to identify this event electrically until the day after the surge, when the resistance values started to return to normal. The Cue ovulation predictor is a device now being marketed based on the work of Albrecht et al. However, since it requires the use of a vaginal probe every day for at least a week, only a highly select, compliant, and well-motivated patient population can use this device.

Combining the work of Burr et al and Albrecht et al, one would conclude that change in vaginal electrical resistance signals impending ovulation, but only vaginal electrical potential clearly identifies the event.

Dermal DC potential measurement is an easy and simple technique which could have broad practical application, as the use of vaginal probes would probably not win wide clinical acceptance. At present the use of basal body temperature for identifying the time of ovulation or the adequacy of the corpus luteum is less than precise. The fallibility of body temperature in determining ovulation has been the subject of several investigations (7, 8). As suggested by the findings for patient 3, the shift in temperature may be out of synchrony with ovulation for several days.

If this is true, the use of basal body temperature to predict the optimal time for coitus may be less reliable than previously believed. Furthermore, changes in sleep patterns and various psychogenic factors will often invalidate the measurement of temperature. Conversely, as long as the DC dermal potential is not determined during a period of acute emotional excitement or physical stress, these variables seem to play an insignificant role in interfering with DC potential results.

Several intriguing questions are raised by the results of this preliminary study. As mentioned, measurements of peripheral electric potentials might well complement or improve on the use of temperature level to pinpoint ovulation.

The precise mechanism for alteration in dermal DC potentials coincident with ovulation is not fully understood. Further work comparing dermal resistance and potential may add to our knowledge, but ultimately we may need to return to the work of Burr et al and use at least ultrasound or even laparoscopy to establish the relationship between the LH surge, electrical signals, and the day, if not the moment, of ovulation.

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Mezlocillin in the Treatment of Acute Salpingitis

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Abstract

Twenty-eight women with laparoscopically documented salpingitis underwent a 5-day course of treatment with intravenous mezlocillin followed by peroral treatment with ampicillin. The most prominent bacteria recovered from the peritoneal cavity of the infected patients were *Neisseria gonorrhoeae* and *Escherichia coli* but anaerobes were also frequently encountered. *Mycoplasma, Ureaplasma,* and *Chlamydia* were not studied. Ninety-three percent of the patients showed an excellent reponse to mezlocillin, and only two patients needed additional antibiotics to recover.

Salpingitis is a common disease which over the last 200 years has been increasing in frequency throughout the world, including the United States (1–3). The treatment of salpingitis has been enigmatic since the goal of treatment has not been clearly determined. Certainly, the direct aim is to decrease signs of the acute infection and make the patient asymptomatic. Also to be considered is long-term treatment success, including elimination of chronic pelvic pain and the avoidance of such sequelae as infertility caused by tubal damage (4).

The treatment of salpingitis has centered on the penicillinlike drugs. Recent awareness of the polymicrobial etiology of salpingitis has resulted in the introduction of multiple antibiotic combinations with complicated administration schedules (4, 5). Introduction of the new broad-spectrum penicillins active against both gram-positive and gram-negative organisms, including anaerobes, has simplified the treatment, but the effectiveness of the treatment in laparoscopically documented cases of salpingitis has not been well described.

In the present study we have evaluated mezlocillin, an acylureido penicillin with an antibacterial activity of a broad spectrum, in the treatment of acute salpingitis (6). The diagnosis of salpingitis was documented in a consecutive series of 28 patients after laparoscopic evaluation of the pelvic organs. The procedure included multiple samplings for microbiologic testing from the pelvic area. It is concluded that mezlocillin has a beneficial effect in the treatment of acute salpingitis.

Materials and Methods

Twenty-eight consecutive patients admitted to the emergency room at Mount Sinai Hospital Medical Center, Chicago, from July 1982 to January 1983 were included in the present series. Mean age was 23.3, with a range of 17–31 years. Informed consent by all participants was obtained in accordance with the hospital's review board criteria. Pregnant patients and patients with a known allergy to penicillin were excluded from the study. Patients entered the study based on two or more of the following clinical criteria suggestive of acute salpingitis: lower abdominal pain, abdominal-pelvic tenderness with rebound

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or ileus, cervical tenderness on motion of the cervix, elevated temperature (over 100.4°F), abnormal vaginal discharge (with respect to amount, color, and odor), elevated white blood cell counts and elevated erythrocyte sedimentation rate (more than 15 mm/hr).

On admission, blood cultures and a cervicovaginal culture for *N. gonorrhoeae* were taken, and patients were then hydrated frequently for a few hours with intravenous fluid prior to being brought to the operating room. An endometrial biopsy as well as an endometrial lavage was obtained through the cervix after the cervicovaginal area was soaked with Betadine solution. The endometrial biopsy was then studied histologically and microbiologically. Thereafter, a routine closed laparoscopy was performed, utilizing a subumbilical incision and a second incision site in the suprapubic area. Upon laparoscopic visualization of the pelvic area, the clinical findings were staged by severity of the inflammatory response into mild, moderate, or severe salpingitis according to criteria established by Hager et al (7).

Bacterial cultures were obtained at the time of laparoscopic evaluation from the tubal ostial area on both sides and from fluid in the cul-de-sac. A gram stain was performed on all peritoneal specimens. All specimens were cultured by the hospital's microbiology laboratories for *N. gonorrhoeae,* as well as aerobic and anaerobic bacteria, according to standard procedures. At the time of this study we were unable to peform cultures for *Mycoplasma hominis* and *fermentans, Ureaplasma urealyticum,* and *Chlamydia trachomatis.*

Once documentation of pelvic inflammatory disease was obtained laparoscopically, the patient was started on mezlocillin (Mezlin), 4 g intravenously every 6 hr, for 5 days, or longer if clinical cure was not obtained. Response to treatment was followed up by evaluation of daily erythrocyte sedimentation rate (ESR) and white blood cell (WBC) counts, clinical examination with temperature recordings and pulse readings every 4 hr, as well as evaluation of disappearance of abdominal pelvic tenderness. Routine blood chemistries for electrolytes as well as renal and liver function were studied every 2–3 days. Generally, after 5 days of intravenous mezlocillin therapy, the patient was discharged on a regimen of oral ampicillin, 500 mg every 6 hr, continued until the patient was clinically normal and the sedimentation rate was normalized (<15 mm). This treatment was usually continued until a checkup 2 weeks later.

If no improvement took place 3 days after the initiation of intravenous mezlocillin therapy, augmenting the regimen with an additional antibiotic was considered. The decision on the type of antibiotic added was based on the sensitivity pattern of the microbial agent isolated from the peritoneal cavity. In one patient, therapy was continued with intravenous gentamicin and in another patient with intravenous cleocin in addition to the mezlocillin therapy.

Results

Of the 28 patients in the present study, 15 were classed as having mild salpingitis, 9 as having moderate salpingitis, and 4 as having a severe form, with pelvic adhesions, tubal obstruction with exudate, and pus in the abdominal cavity.

Four patients had an IUD in place at the onset of salpingitis. One of the 4, who had moderate salpingitis, had a Lippes loop, and 3 had a Cu-7—1 with severe salpingitis and 2 with moderate salpingitis. All IUDs were removed at the surgical procedure.

The predominant bacteria isolated from the endocervical, endometrial, and peritoneal cavities in these patients were *N. gonorrhoeae* and *E. coli* (Table). All blood cultures were negative.

Of the 28 patients, 24 (85%) responded to mezlocillin therapy with a dramatic improvement in the physical findings of tenderness, temperature, and pulse rate. Two patients (7%) required a course of mezlocillin treatment longer than 5 days to normalize the physical findings, and 2 patients were given additional antibiotics.

Parenteral administration of mezlocillin was well tolerated. One patient developed superficial thrombophlebitis at the intravenous infusion site. No hepatic or renal toxicity was encountered during mezlocillin therapy.

The WBC count (Fig. 1) was reduced to normal levels within 3–4 days after initiation of mezlocillin therapy. This reduction took place concomitantly with the disappearance of patients' symptoms. In contrast, the ESR (Fig. 2) increased markedly from the day of admission/laparoscopy for 1–2 days after initiation of treatment, remained quite elevated for the entire mezlocillin treatment period, and showed no clear decline 2 weeks later.

In follow-up by physical examinations of the patients for up to 6 weeks after the therapy was started, findings were normal except in one patient, who had moderate abdominal pain probably related to pelvic adhesions.

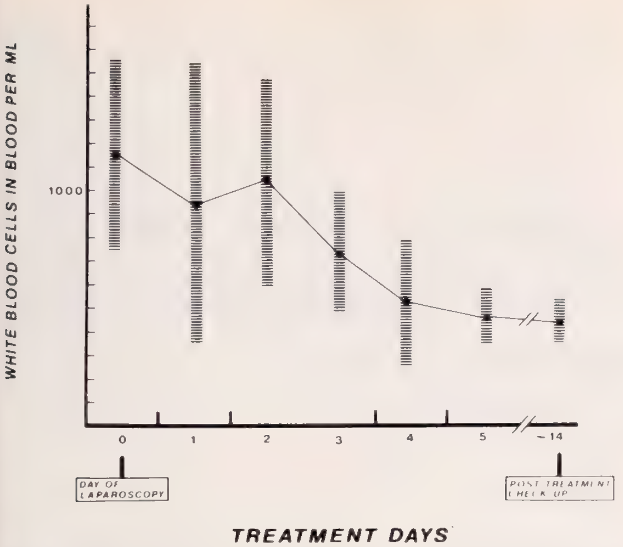
TABLE 1
Bacteriologic Findings in 28 Patients with Salpingitis

Pt. no.	Age	Laparoscopic staging	Bacteriologic findings on specimens from peritoneal cavity
1	25	Moderate	*Bacteroides* sp.
2	19	Moderate	No growth
3	21	Moderate	*Staphylococcus aureus*
4	20	Moderate	No growth
5	26	Mild	*E. coli*
6	31	Mild	*N. gonorrhoeae*
7	21	Moderate	β-Hemolytic streptococci group B *N. gonorrhoeae*
8	18	Severe	*N. gonorrhoeae*
9	17	Mild	No growth
10	17	Mild	No growth
11	22	Mild	*Peptostreptococcus* sp.
12	29	Mild	No growth
13	28	Mild	*Bacteroides* sp. *Klebsiella pneumoniae*
14	30	Moderate	No growth
15	20	Moderate	*E. coli* *N. gonorrhoeae*
16	27	Severe	No growth
17	19	Mild	*E. coli* *Peptostreptococcus* sp.
18	21	Severe	*N. gonorrhoeae*
19	17	Moderate	No growth
20	25	Mild	No growth
21	28	Mild	No growth
22	29	Moderate	*Staphylococcus epidermidis*
23	25	Mild	No growth
24	18	Severe	No growth
25	25	Moderate	*Bacteroides* sp. *E. coli*
26	23	Moderate	*N. gonorrhoeae*
27	25	Mild	No growth
28	21	Mild	No growth

Discussion

The degree of therapeutic success reported in the treatment of salpingitis is dependent on the parameters studied. In the present study the return to normal physical findings was excellent in 93% of the patients and slower in 7% of the patients. All patients, however, were more or less symptom-free after 5 days of intravenous antibiotic therapy. Only one patient had a noticeable physical impairment 6 weeks after the acute onset of the disease. This good response to intravenous administration of mezlocillin confirms previous reports of successful treatment of intraabdominal infections with intravenous mezlocillin (8–10). The decrease in WBC counts accompanied recovery of the patients, and in the majority of the patients the physical findings were normalized after 2–3 days of therapy. The fact that the ESR continued on an upward trend indicates that the inflammatory reaction in the tubes remained and suggests that prolonged antibiotic treatment is advisable; intensive intravenous infusion of antibiotics is probably not necessary, and peroral administration of antibiotic treatment on an outpatient basis may be sufficient.

The bacteriologic findings in different study populations probably depend on the selection of patients. The most prominent bacteria cultured in the present series was *N. gonorrhoeae,* which thus for our study population is a prominent cause of salpingitis. In 14 patients, in spite of clinical and laparoscopic signs of salpingitis, no positive bacterial growth from the peritoneal washings was obtained. During this study we did not have any facilities to perform cultures for *Chlamydia, Mycoplasma,* or *Ureaplasma;* some of the culture-negative patients may have had infections of *Chlamydia trachomatis, Mycoplasma,* or *Ureaplasma*. The isolation of *Staphylococcus*



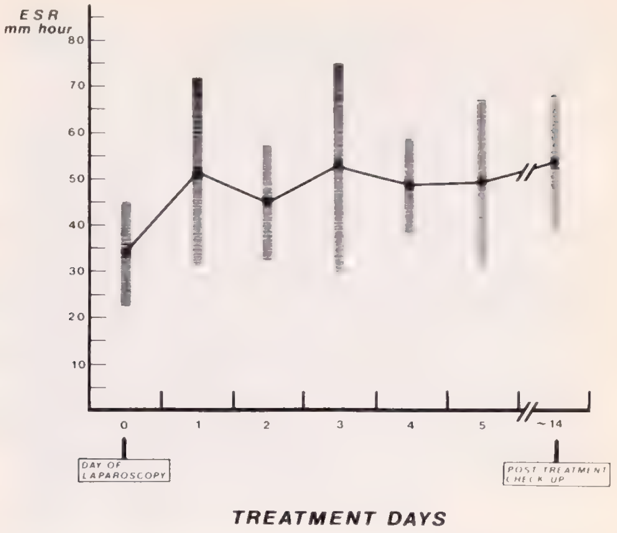


Fig. 1. Left Daily changes in number of white blood cells on day of laparoscopy and subsequent 5 postoperative days and at 2-week follow-up.

Fig. 2. Right Daily changes in erythrocyte sedimentation rate (ESR) on day of laparoscopy and 5 subsequent postoperative days and at 2-week follow-up.

epidermidis from the abdominal cavity could probably be related to contamination from the abdominal skin incision. The findings of anaerobes in the abdominal cavity, however, is clearly significant and indicates that at least in some of the patients these bacteria played a significant role as etiologic agents (4, 5).

Intravenous administration of mezlocillin resulted in clinical improvement in a large proportion of patients. A few patients, however, required additional antibiotics to obtain a satisfactory clinical response. The disappearance of fever and the resolution of abnormal clinical findings in the majority of our patients indicate that intravenous administration of mezlocillin was an appropriate first-treatment choice for a large number of our patients with salpingitis.

We consequently conclude that intravenous administration of mezlocillin in the treatment of salpingitis is effectively our drug of first choice in an inner-city population severely afflicted by pelvic inflammatory disease. For a few patients, additional medication will become necessary for total clinical resolution of the disease. The number of patients requiring additional therapy is, however, so small that cost-benefit considerations would not warrant a multiple-drug approach to the patient with uncomplicated salpingitis. Our study thus confirms some previous reports indicating that a single broad-spectrum antibiotic such as mezlocillin is an appropriate choice as a first drug in the treatment of salpingitis. If no clinical response is obtained within 48–72 hr, additional antibiotic coverage, primarily for anaerobic bacteria or *Mycoplasma, Ureaplasma,* and *Chlamydia,* is to be instituted (9, 10).

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Hepatic Ectopic Pregnancy: Successful Surgical Treatment of a Patient with Hepatic Pregnancy and Acute Hemorrhage

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Abstract

A patient with hemoperitoneum and shock was examined and operated upon with the diagnosis of ectopic pregnancy. At laparotomy, a hepatic pregnancy was found and successfully treated by excision. This is reported as the second surgically treated patient with hepatic pregnancy and the first to be successfully operated on during an acute hemorrhagic episode.

Ectopic pregnancy has been less frequently observed in emergency wards during recent years, probably because of anovulatory medication and the use of other contraceptive measures. However, an increased incidence of extrauterine pregnancies in women using an intrauterine device is being reported in the medical literature.

We are aware of only two cases of hepatic pregnancy previously reported. One of them was quoted by Queiroz (1), who presented a picture of a liver showing hepatic ectopic pregnancy found at autopsy at the Medico-Legal Institute in Rio de Janeiro. The other patient, reported by Gordeev (2), was a 26-year-old white woman with abdominal epigastric pain, loss of weight, and general malaise, seen in February 1978 with a history of bloody discharge from the vagina. A diagnosis of dysmenorrhea was made and she was subjected to dilatation and curettage. She had had only one child and no abortions, and her last coitus was said to be in 1975. Further investigation resulted in a revised diagnosis of echinococcosis of the liver. She was operated upon on February 17, 1978. A tumor on the left lobe of the liver, which yielded dark-brown fluid on puncture, was found. Dissection of the tumor revealed a cyst with masses of brown liver detritus and a vesicle in which there was a 16-week-old female fetus, connected by an umbilical cord implanted into the depth of the cyst. Dissection of the placenta gave rise to profuse arterial bleeding resulting in cardiac arrest, which was treated successfully by resuscitation and surgical hemostasis. The cavity was packed and the tamponade was removed within three weeks.

Alcalay et al (3) reported a case of splenic pregnancy with survival following splenectomy. Their review of the literature revealed only four such cases of this very unusual location of an ectopic pregnancy.

The following report is the third case known to us of hepatic ectopic pregnancy, and the first successfully treated by surgery during an acute hemorrhagic episode.

Case Report. A 27-year-old married white woman was admitted to the private practice of the senior author at Hospital São José, Rio de Janeiro, Brazil on June 18, 1983 with the chief complaint of sudden abdominal pain and fainting followed by shock with nonpalpable radial pulse

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and undetectable blood pressure. She was immediately admitted and resuscitated by rapid infusion of intravenous normal saline.

The patient's last normal menstrual period had been 38 days before. She had an IUD in place for the last 5½ years. She had had two normal pregnancies, with a normal birth 4 years and 9 months before and a cesarean section and myomectomy 1 year and 9 months before. Her only other previous surgery was a mammoplasty 12 years before. The patient complained of amenorrhea of 10 days' duration as well as slight vaginal bleeding on the day of her expected period.

On physical examination the patient showed intense skin pallor with discolored mucosae, thin radial pulse (140 bpm), and a blood pressure of 70/0 after the initial resuscitative measures. She was alert but feeling very weak. There were well-healed scars of the previous breast surgery and a slightly hypertrophic, well-healed Pfanenstiel incision in the lower abdomen. Palpation of the belly disclosed generalized tenderness and rebound tenderness. The abdomen was distended with diminished bowel sounds. On pelvic examination, the uterine cervix was soft and very tender to lateral movements. The cul-de-sac was very tender to palpation. Abdominal paracentesis yielded blood. The rest of the physical examination was normal.

Laboratory investigation revealed a hematocrit of 31%. A blood transfusion was started, and she was immediately taken to the operating room with the diagnosis of ectopic pregnancy, probably tubal.

Laparotomy was performed by excising the previous Pfanenstiel scar. The uterus was soft, enlarged, with signs of early pregnancy. Both tubes and ovaries were normal. There were small hemorrhagic cysts on the ovaries bilaterally, which could not account for an enormous amount of clotted and unclotted blood found in the abdominal cavity, roughly calculated as 3,000 ml. Careful examination of the anterior and posterior surfaces of the uterus did not show perforation by the IUD.

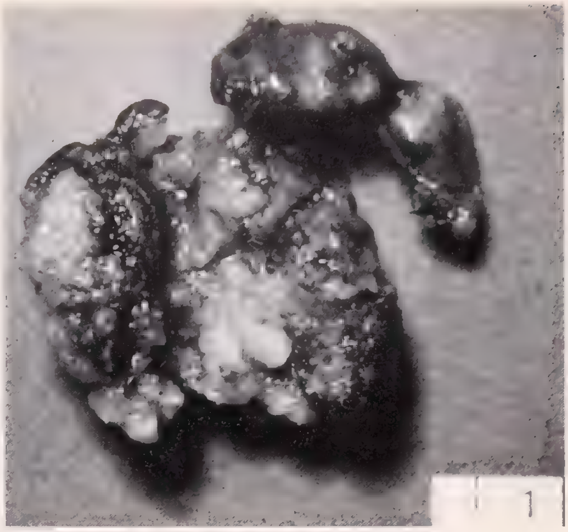

Fig. 1. Specimen measuring 3 × 2 × 1.5 cm showing liver fragment in right upper corner. Below, placental tissue.

A right paramedian supra- and infraumbilical incision was immediately performed for exploration of the rest of the peritoneal cavity. The liver, spleen, stomach, duodenum, pancreas, main abdominal vessels, mesentery, small and large bowels, and kidneys appeared normal. After all blood had been aspirated from the abdomen, a small soft tumor was palpated at the inferior surface of the liver at the lower border, near the right kidney. This mass, measuring about 2 cm, was bleeding profusely. With a tenta-

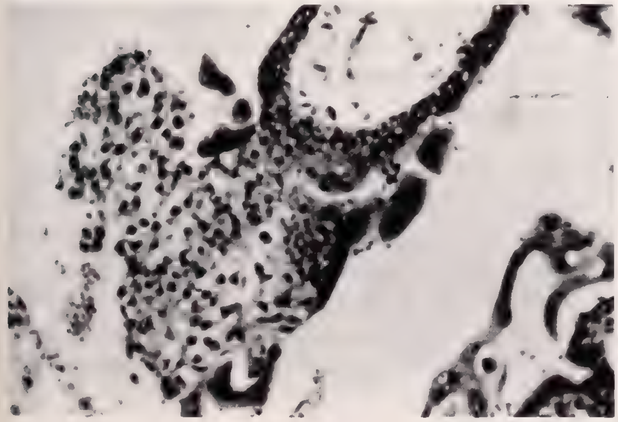

Fig. 2. Chorionic villi and trophoblastic tissue.

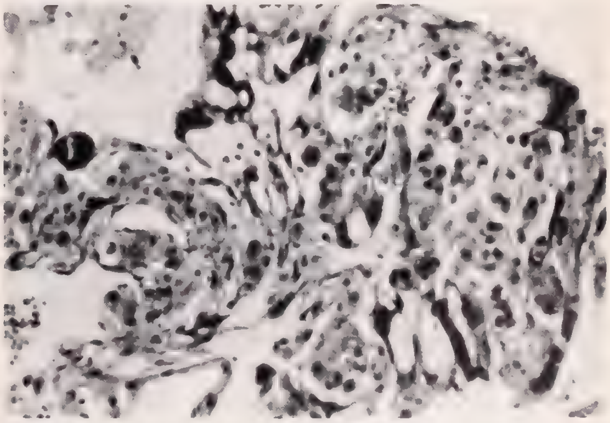

Fig. 3. Chorionic villus and capillaries with red blood cells.

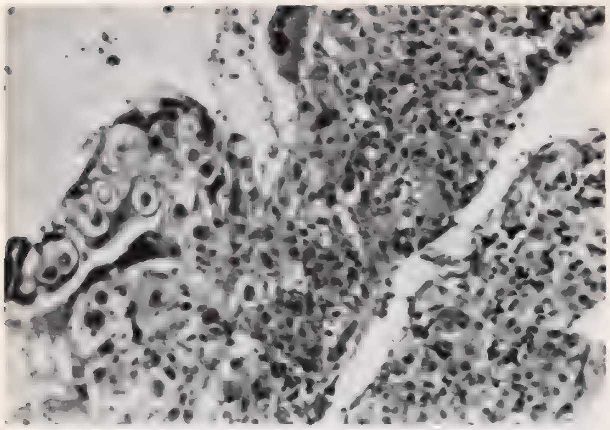

Fig. 4. Hepatic tissue with trophoblastic cells within sinusoids.

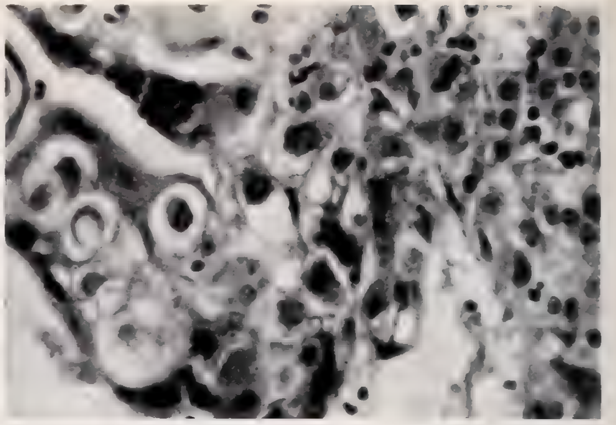

Fig. 5. Higher power of field in Fig. 4.

tive diagnosis of ruptured hepatic hemangioma, excision was undertaken as a small partial hepatectomy. Hemorrhage ceased. The patient had received 4 units of whole blood during the operation.

Both abdominal incisions were closed with figure-of-eight polyglycolic acid sutures, and the skin was closed without drainage with 3-0 cotton stitches.

Postoperatively the patient received 2 more units of blood, and she was discharged subsequently from the hospital without complication on the fifth postoperative day.

The pathology report showed ectopic pregnancy, hepatic in origin. The specimen consisted of 3 × 2 × 1.5 cm irregular, whitish, friable tissue, with dark-brown areas of spongelike tissue (Fig. 1). Microscopic examination showed the greatest portion of the tissue consisted of large chorionic villi, with a double trophoblastic lining, a loose stroma, and capillaries that occasionally contained intraluminal red blood cells (Figs. 2, 3). The adjacent hepatic tissue showed periportal nuclear infiltrates and dilated sinusoids with trophoblastic cells (Figs. 4, 5).

Discussion. Ectopic pregnancy, less frequent today owing to the widespread use of anovulatory drugs, is an abnormality that occurs frequently in a busy emergency ward in any hospital. Ringrose (4) has reported an increased incidence of nontubal ectopic pregnancies in women using an intrauterine device. Modern medicine has provided us with a means of decreasing the incidence of pregnancies (the anovulatory drugs), but has also, by reports such as the one quoted above, given us indications of new pathologies that are now being seen more frequently, sometimes very dangerous and often difficult to diagnose and treat.

The patient reported by Gordeev (2) disclosed an unusual occurrence: growth of the fetus with maceration and cyst formation, persistent without symptoms for about 3 years after nidation of the ovulum in the hepatic tissue. The senior author has treated two similar patients. One had a bilateral tubal pregnancy, ruptured on the left with a macerated, nonbleeding fetus in the right tube. The other patient had an abdominal mass compressing the cecum. This mass was a right tubal pregnancy, adherent to the right pelvic wall and compressing the cecum.

We have reported here on the patient we treated because of the rarity of the case—the first reported case in the medical literature successfully treated during an acute hemorrhagic episode. Another point worth emphasizing is the necessity for general surgical training of gynecologic and obstetric surgeons. With increasing incidence of ectopic nontubal pregnancies, a surgeon may be faced with the immediate indication for a lifesaving, difficult splenectomy or partial hepatectomy. General surgical training is essential for all specialized surgeons and should be available in all universities and teaching hospitals.

The growth of trophoblasts into the liver tissue gives rise to profuse bleeding, as occurred in the reported patient. The question of diagnosis is vital. Hepatic ultrasonographic examination and/or computerized axial tomography should be critical in recognizing ectopic pregnancy and permit prompt intervention before hemorrhage and shock supervene and result in morbidity if not mortality.

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Minilaminotomy, an Alternative to Microdiscectomy or Standard Laminectomy

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Abstract

Two neurosurgeons in suburban practice performed a series of 200 minilaminotomies. Simple positioning of the patient flexed prone on bolsters allowed intraoperative as well as preoperative confirmation of the correct level. The incision was 4 cm in length, and only the Taylor retractor and standard instrumentation were employed. A fiberoptic head light and 4.5 × binocular loupes provided adequate illumination and magnification of the spinal canal, neural elements, epidural veins, adhesions, extruded fragments, osteophytes, and the disc space itself. Removal of degenerative disc material with pituitary rongeurs yielded 6–8 gm of specimen. The overall results were comparable to reported series of microdiscectomy: minimal blood loss, less narcotic requirement, fewer days in the hospital, earlier return to normal activity, no wound infection or discitis, and low rate of second surgery.

Williams (1) presented his original concept of microlumbar discectomy at the Congress of Neurological Surgeons in Ponte Vedra Beach, Florida in 1975. Goald (2) restudied the technique in 1978 and similarly advocated (a) preoperative x-ray localization with as small a skin incision as possible, (b) magnification and illumination provided by the operating microscope, (c) no alteration of the lamina or facet joint, (d) no coagulation or removal of fat within the epidural space, and (e) removal of a minimal volume of disc material without incision of the annulus or curettage. Subsequent clinical investigation (3–6) concluded that each of the above principles alone might be responsible for results superior to standard laminectomy. At one university hospital (7), the patient was placed in a lateral oblique position so that the surgeon could sit while using the operating microscope to perform a microdiscectomy. After hearing multiple requests by patients for microscopic surgery, two neurosurgeons working at two community hospitals performed a series of 200 minilaminotomies.

Methods

All of the patients referred for surgery had radicular pain unrelieved by conservative treatment for at least 6 months, or objective findings on neurological examination, such as motor or reflex deficits, or both. CT scans (Fig. 1) and myelography (Fig. 2) were performed in every case. Preoperative review of the neuroradiologic studies accurately predicted the operative findings and often dictated medial facetectomy or foraminotomy for relief of segmental spondylosis or lateral recess syndrome.

The patient was positioned prone on bolsters, and the operating table was maximally flexed (Fig. 3). One gram of cefazol was administered intravenously as antimicrobial prophylaxis. The 4-cm incision was midline and dictated by an

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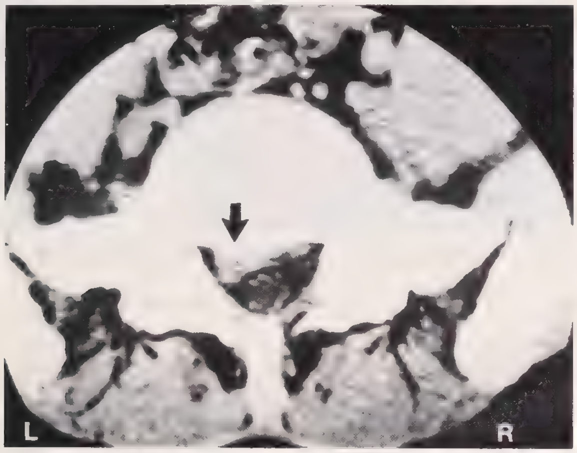
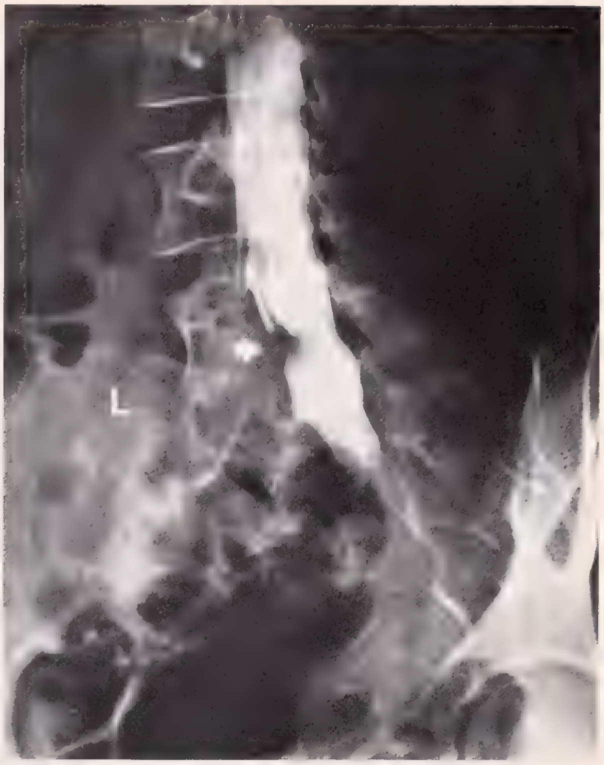
Fig. 1. Left CT scan of L5-S1 disc level with thin-slice magnification technique. Arrow shows area of extruded disc fragment.
Fig. 2. Right Right anterior oblique view of lumbar myelogram with metrizamide. Arrow indicates filling defect consistent with herniation of the disc at L5-S1 level.





x-ray with a spinal needle in place (Fig. 4). Hemostats were placed on the cuticular edges, and a small self-retaining retractor was applied to the wound. Fat was obtained from the subcutaneous layer and saved for grafting. The deep fascia and paravertebral muscle were incised and scraped from their attachment to bone with periosteal elevators. A Taylor retractor, hooking on the facet, provided adequate retraction of the incised muscle and fascia. The exposed spinous processes and laminae were cleansed with gauze abrasion until the ligamentum flavum was well visualized. Bleeding was controlled initially with a suction Bovie instrument, but as soon as the ligamentum flavum was opened and resected only the Malis bipolar coagulator was employed.

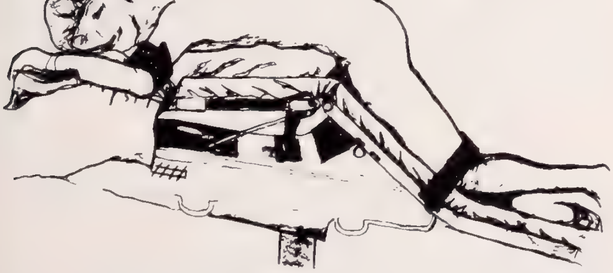
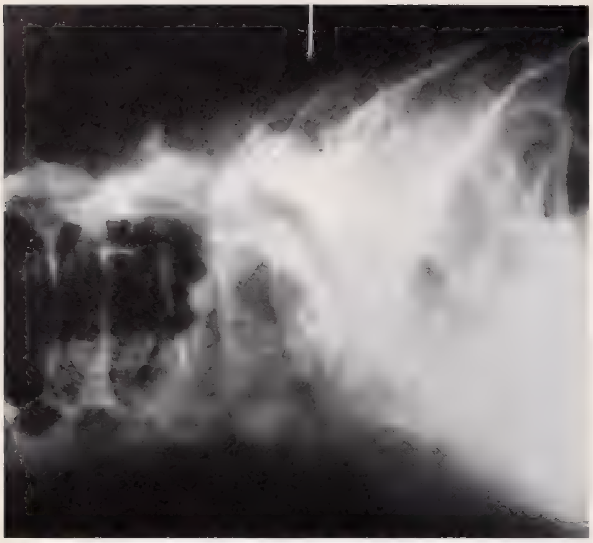


Fig. 3. Artist's drawing of prone positioning of patient on bolsters made of rolled blankets. X-ray cassette is in place.

A second x-ray was taken with a sterile casette cover to confirm the level of exploration and to show the relation of the interlaminar approach (Fig. 5). After the neural elements were protected by a cottonoid sponge, laminotomy, as well as medial facetectomy and foraminotomy if indicated, was performed with Kerrison punches (Fig. 6). The nerve root and dura were then retracted gently toward the midline. Bleeding epidural veins and inflammatory adipose tissue were coagulated with the Malis bipolar instrument. Any adhesions noted were lysed. When an extruded fragment or protruding portion of disc was found, the degenerative material was excised by a no. 11 blade on a long handle. If the disc space was to be explored directly, a 2 mm × 4 mm window was cut in the annulus fibrosus. Pituitary rongeurs with straight, up-cutting, and down-cutting angles were used to grasp fragments of nucleus pulposus and to empty the disc space. Curettage of the cartilaginous plates was specifically avoided. About 6–8 gm of disc material was regularly recorded on the pathology report.

Prior to closure, the nerve root was carefully inspected to verify the absence of any compression. The operative field was copiously irrigated with saline. Meticulous hemostasis was accomplished before coating the neural elements with the fat tissue obtained during the opening. Gelfoam was also applied to the area of laminotomy.



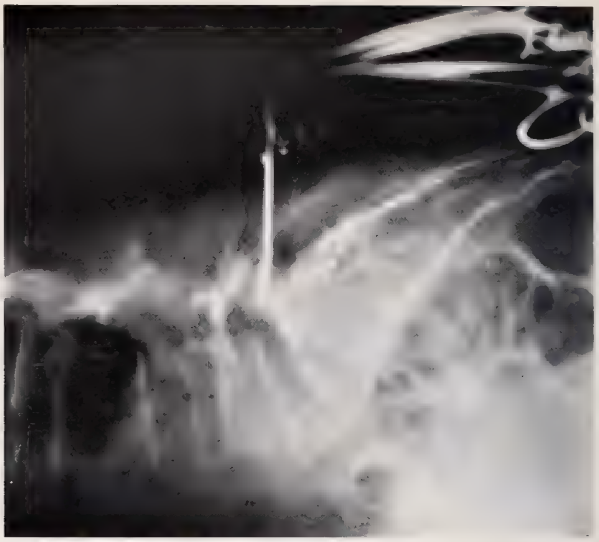


Fig. 4. Left Preoperative lateral view of lumbosacral spine taken on the operating table to determine incision. Note shadow of spinal needle over L5-S1 interspace.
Fig. 5. Right Intraoperative lateral view of lumbosacral spine, confirming level of exploration and determining relative position of L5-S1 interspace.

The retractors and hemostats were removed, and the wound was closed with interrupted sutures for all layers. The total operating time, including the development of x-rays, was 45 to 60 minutes for one level, and 60 to 90 minutes for 2 levels when a 5-cm incision was made.

The patient was allowed a bedside commode immediately postoperatively and not restricted as to diet. Initially morphine was prescribed, but an oral pain medication was ordered on the second or third hospital day. Recovery in the hospital following minilaminotomy never exceeded one week, and most patients were discharged two to four days after surgery. Skin tapes were applied to the incision after removal of the sutures. Advice was given about performing normal activity, showering, walking, and avoiding prolonged sitting. A follow-up visit was scheduled after 3 weeks of convalescence at home. Instruction in exercises for the lower back was offered, and regular evaluation was done at 3-month intervals for at least one year.

Results

The ages of the patients ranged from 18 to 72. A total of 112 men and 88 women came to surgery; 36% were compensation cases. The L5-S1 disc was herniated in 116 patients, the L4-5 disc in 68 patients, the L3-4 disc in 3 patients, and both L4-5 and L5-S1 in 13 patients. No complication of increased neurological deficit, wound infection, discitis, cerebrospinal fluid fistula, or hemorrhage requiring transfusion occurred in our series of 200 minilaminotomies. Two cases of thrombophlebitis were diagnosed; readmission and prolonged hospitalization for anticoagulation therapy were prescribed without further complications.

Preoperatively, all of the patients were confined to bed for extensive periods by sciatica and muscle spasm. Postoperatively, every patient was ambulatory and able to be evaluated at an office visit. In each case, the question was asked, "Are you better?"; 185 replied affirmatively, and 15 said, "No."

The patients who were not improved were reevaluated by CT scans; the neuroradiologic imaging revealed postoperative changes at the correct level. Five patients were reexplored; the fat graft made the dissection technically simple. Uniformly the operative findings were scar tissue arising from the area of incised annulus and attached to the nerve root. Lysis of the adhesions was performed, but only 3 patients reported diminished radicular pain. The other 10 patients underwent radiofrequency facet rhizotomy (8), and 5 improved. Four of the patients who failed to gain any relief of pain were diabetic.

Discussion

Positioning of the patient either laterally or obliquely does not allow intraoperative confirmation of the disc exploration; with only preoperative x-rays, the correct level may be missed (1). Paramedian incision was stressed by Wilson and Harbaugh (3) for placement of the Williams re-

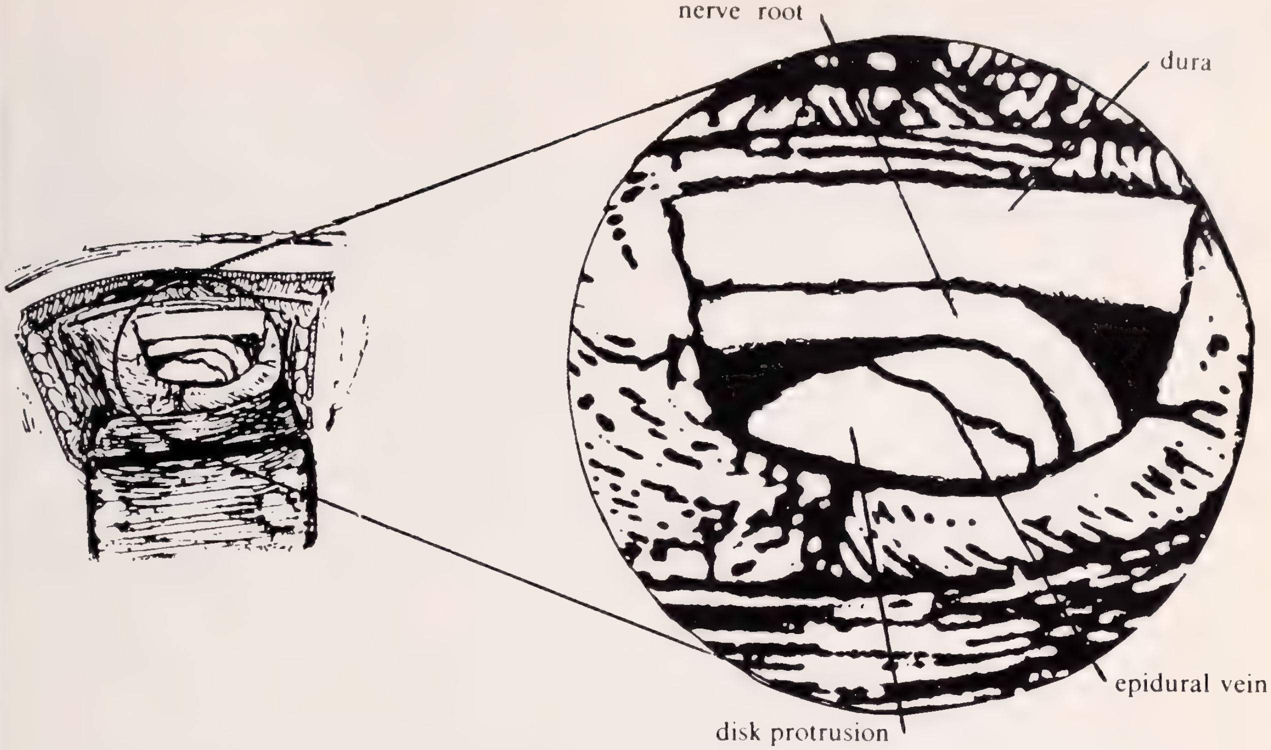


Fig. 6. Artist's drawing of minilaminotomy incision with surgeon's view magnified 4.5× by binocular loupes.

tractor, and by Balagura (5) to avoid cutting the supraspinal ligament. Our own incision was midline and 4 cm in length rather than 1 inch. An ordinary Taylor retractor hooking on the facet provided complete retraction of the paraspinal muscle and fascia.

No attempt was made to avoid removal of bone or ligamentum flavum as suggested by Williams (1); standard Kerrison punches were not abandoned in favor of a high-speed drill. Laminotomy was necessary in most cases to visualize the superiorly positioned disc space. Medial facetectomy and foraminotomy not only relieved segmental spondylosis and lateral recess syndrome but facilitated removal of extruded fragments without excessive nerve root retraction. As for the epidural fat and veins, hemostasis and clear inspection of the posterior longitudinal ligament were the prime considerations.

Williams (1) stressed separating the fibers of the annulus fibrosus with a Penfield dissector and removal of a minimal amount of nucleus pulposus in order to allow the ligament to heal. In our own cases, the annulus was regularly incised with a no. 11 blade to create a 2mm × 4mm window; and the disc space was emptied of 6–8 gm of degenerative material with standard pituitary rongeurs. Not scraping the cartilaginous plates with curettes may lessen the chances of discitis.

Elaborate methods of assessing the results of lumbar disc surgery have been described (9). Questionnaires, psychological testing, serial neurological examination, the bias of compensation cases, electromyographic testing, and computer analysis have all been applied. Our own way was quite direct, simply allowing the patient to judge.

Summary

As early as 1963, Nachemson and Hirsch (10, 11) advocated small laminotomy incisions with limited removal of only the degenerative or completely herniated disc fragments and credited the concept to several others, including Love (12), who published in the Proceedings of the Mayo Clinic in 1939. The surgical technique of minilaminotomy should be easily mastered by neurosurgeons performing standard laminectomy for removal of a herniated lumbar disc. Since ordinary instruments are employed, a fiberoptic headlight and binocular loupes are the only other equipment needed. Even when an operating microscope is available, a fully trained microneurosurgeon may prefer the speed and ease of using a coaxial beam of cold light with 4.5× magnification. The overall results of minilaminotomy compare favorably to results in other published series of microdiscectomy (1–7).

Acknowledgments

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Percutaneous Radiofrequency Rhizotomy of the Lumbar Facets

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Abstract

Two neurosurgeons in suburban practice performed a series of 115 percutaneous radiofrequency facet rhizotomies on patients with incapacitating low-back pain after various modalities of conservative treatment had failed. Neuroradiologic studies were negative or failed to reveal a herniated disc in all patients; 56 patients had undergone previous spinal surgery, and 59 had lumbosacral radiculitis. The combined use of fine thermocouple electrodes, image intensifier, nerve stimulator, and radiofrequency lesion generator provided a relatively simple and safe technique for the relief of pain. The procedure was performed with the patients under local anesthesia so that the patient could respond to continuing testing. Control of the temperature over the articular nerve roots permitted selective denervation of the pain conduction fibers. In 66% of the patients, marked improvement was noted in terms of reduced medication requirement and increased employment; in 34% there was no essential change.

In 1971, Rees (1, 2) reported on multiple bilateral percutaneous rhizolysis of the nerves of the zygapophyseal joints for relief of intractable low-back pain. King (3) suggested that the operation was in fact a myofasciotomy rather than a neurectomy because Rees's blade (1, 2) was not long enough to reach the facet joints. Shealy (4, 5) performed percutaneous rhizotomies of the articular nerves of Luschka with a radiofrequency thermocoagulator; placement of the electrode was confirmed by intraoperative fluoroscopy. This benign percutaneous technique produced symptomatic relief in enough patients to merit further clinical investigation.

Methods

Fifty-six patients who had undergone prior spinal surgery developed recurrent debilitating low-back pain; an additional 59 patients developed the signs and symptoms of a herniated disc. There were 67 women and 48 men in the study; their ages ranged from 18 to 72 years. In all patients, the neuroradiologic studies were negative or failed to confirm herniation of the nucleus pulposus. None of our patients had a narrow vertebral canal or spinal instability requiring surgery. Each patient was evaluated by CT scan and myelography or magnetic resonance imaging. Every patient's condition had, over an extended period, been refractory to management by conservative measures including trials of bed rest, traction, back brace, low back exercises, antiinflammatory agents, analgesics, muscle relaxants, physiotherapy, and transcutaneous electroneural stimulators.

The patient was positioned prone on a radiolucent table compatible with the C-arm image intensifier; posteroanterior and lateral views were readily obtained. The low back was prepared and draped in the usual manner. Sterile safety pins placed on the skin aided in identifying the L3-4, L4-5, and L5-S1 levels on both sides (Fig. 1). Xylocaine was infiltrated locally prior to making six 3-mm incisions; no. 14 gauge needles were then inserted to act as guides for the thermocoagu-

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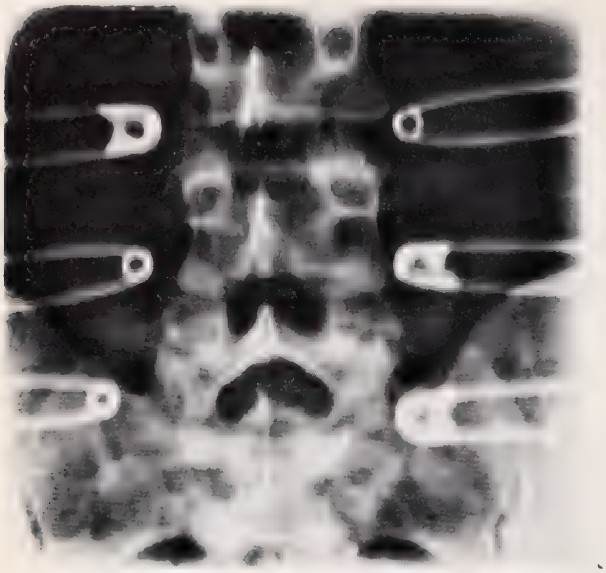

Fig. 1. Posteroanterior x-ray of the lumbosacral spine with safety pins used as markers to locate the facet joints.

lating electrode. The x-ray landmarks, as described by Fox and Rizzoli (6), were the lateral margin of each joint at the junction of the superior third and the inferior two-thirds (Fig. 2). The electrode was placed in final position by the maneuver of walking off the lateral margin of the facet.

The system utilized is commercially available from Radionics (model RFG 3AU). The stimulation mode was set at the following parameters: 0–10 volts, 50 Hz, and 1 millisecond pulse width with square wave. Either droperidol or diazepam was given intravenously; the patient was still able to answer questions when preliminary nerve stimulation was employed. Testing ascertained that the electrode was not too close to the anterior primary ramus and often reproduced the patient's initial pattern of pain.

Because of the unpredictable quality of referred pain, denervation was attempted at the lower three facets on both sides. Three separate lesions were made at each of the six facets to straddle the articular nerve. The lesion mode was set for 50 seconds, and the temperature was maintained at 80°C by thermocouple monitoring. Superior and caudal positioning of the electrode was accomplished by simply pushing on the exposed end of the guide needle. The area of electrocoagulation was planned to be 10 mm in diameter.

Patients were allowed to move to the bathroom and had no diet restrictions. All but three patients were discharged for return home the day following the operation. Office visits at extended intervals provided follow-up of the relief of pain. Every patient was followed up for at least one year before being included in the study. Response to the procedure was graded at four levels:

0 minimal subjective improvement.
1 activities of daily living required medication.
2 engaged in all activities except work.
3 resumed employment.

Results

Seventy-six patients (66%) achieved a response at level 2 or 3. When ongoing litigation was considered, the success rate fell to 61%, versus 76% for those not involved in compensation or liability cases. The procedure was more beneficial (75%) in patients who had no prior surgery; far fewer patients (58%) with prior laminectomy improved. Four patients who achieved a response at level 3 later required repeat facet rhizotomy after more than one year of relief, and three were able to return to work.

In two of the patients who had had no prior surgery and who obtained little relief from the procedure (level 0 and 1), two complications were recorded. A 58-year-old man suffered a 1-cm diameter burn which required only local treatment with zinc oxide ointment. A 62-year-old woman developed herpes zoster in the lumbosacral area which was treated with acyclovir ointment; she subsequently attended a pain clinic and achieved a level 2 response. Two patients who had previously undergone spinal surgery were reexplored for lysis of adhesions after unsuccessful facet rhizotomy; both their responses remained at level 1.

Discussion

Shealy (4, 5), working at a pain rehabilitation center, treated more than 300 patients with chronic back pain and sciatica by percutaneous radiofrequency facet rhizotomy. He believed that much of the pain associated with low-back syndrome, lumbosacral strain, degenerative disc disease, herniated nucleus pulposus, and unsuccessful laminectomy arose from the articular facets rather than compromised nerve roots alone. Postoperative relief of pain by lateral spinal fusion might be explained on this basis. Evidence has also been presented earlier that mechanical derangement of the zygapophyseal joints can produce symptoms of sciatica (7–9). During preliminary stimulation of the nerves of

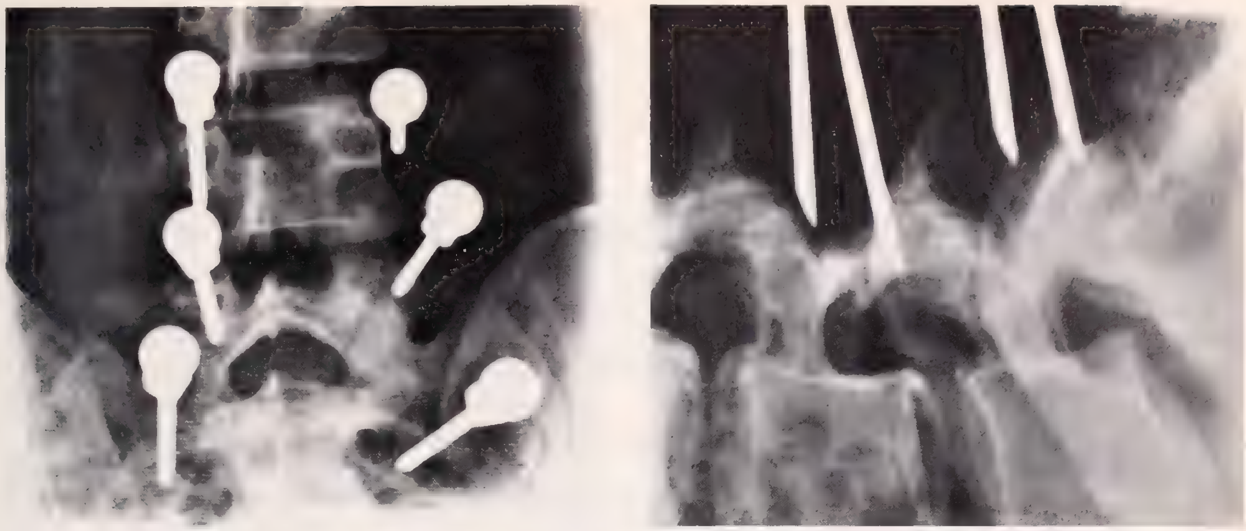

Fig. 2. Posteroanterior (**left**) and lateral (**right**) views of the no. 14 gauge needles employed as guides for the thermocoagulating electrode.

Luschka, most of our patients described referred pain radiating down the posterior thigh.

Lora and Long (10) attempted denervation of the articular facets with radiofrequency coagulation in over 200 patients with intractable spinal pain who were admitted to a university hospital. All of the patients had obtained temporary relief from local anesthetic blockade of the zygapophyseal joints. As reported in Shealy's series (4, 5), the success rate was highest in patients not previously operated on and fell precipitously in patients who had undergone laminectomy and spinal fusion.

In suburban practice, low-back pain is one of the most common problems requiring neurosurgical consultation. The conservative management of lumbosacral radiculitis when a frankly herniated disc is not disclosed on neuroradiologic studies may be unrewarding. Referral to a pain clinic or major medical center is often not desirable to the incapacitated patient. Our own experience at a community hospital with percutaneous rhizotomy of the lumbar facets compares favorably with the previously published results at a pain rehabilitation center (4, 5) and a university hospital (10).

Summary

In selected cases of debilitating low-back pain, percutaneous radiofrequency facet rhizotomy can improve patients previously considered untreatable. One-third of our cases were able to resume employment. Another one-third could markedly reduce medication and engage in the activities of daily living in comfort. The morbidity is quite low, and the hospital stay short. The procedure can readily be performed at small community hospitals.

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Rupture of Allograft Kidney: A Salvageable Condition

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Abstract

Five cases of renal allograft rupture are presented. Salvage was achieved in 2 cases. Pathological studies demonstrated acute tubular necrosis and mild to moderate rejection in all specimens.

Renal allograft rupture has become a frequently recognized event. It usually presents with sudden onset of pain at the graft site, hypotension, fall in hematocrit, palpable mass at the graft site, and occasionally blood at the drain site.

Early surgical intervention is generally indicated to control bleeding, prevent damage to the graft, and alleviate symptoms of pain as well as peritoneal symptoms caused by pressure and irritation. Furthermore, operative intervention will allow biopsy and evaluation of the extent of graft rejection, to assess for potential graft salvage.

We have reviewed cases of graft rupture at The Mount Sinai Hospital in an attempt to identify risk factors and evaluate therapy for this complication of renal transplantation.

Materials and Methods

All transplants performed at The Mount Sinai Hospital between January 1979 and June 1983 were reviewed. Eighty-two renal transplants were performed during this period. These included 63 cadaver-donor and 19 living-donor renal transplants.

Five cases of ruptured grafts were identified. All of these occurred in recipients of cadaver kidneys, with a 5.2% incidence in all transplants and 7.9% in the cadaver-donor transplants. We have reviewed these 5 cases with emphasis on the method of preservation, the age of the graft at time of transplant, the hospital course prior to rupture, histologic appearance of the kidney and treatment.

Case Reports

Case 1. A 35-year-old man was on hemodialysis for two and a half years for renal failure of unknown etiology. The patient received a cadaveric graft with two arteries on an aortic cuff and a polar artery that had been ligated at the time of harvesting. Ligation of the polar artery resulted in a small upper pole area poorly perfused at the time blood flow was reestablished. The graft had been preserved on ice for 24 hr at the time of transplant. A biopsy was taken 1 hr posttransplant.

Postoperatively, the patient required hemodialysis for acute tubular necrosis (ATN). On the ninth postoperative day, the patient developed a painful, tender mass at the graft site, hypotension, fall in hematocrit, and bleeding from the drain site. On exploration, a rupture was found on the anterior lateral aspect of the graft, and 500 cc of blood was drained. The rupture was oversewn with a free muscle patch, and the graft was wrapped in Gelfoam. At surgery the biopsies indicated ATN and mild cellular and vascular rejection.

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The patient was then treated with high-dose oral steroids for rejection. On the seventh day after exploration, diuresis began and renal function improved. The patient was discharged one month posttransplant, with a creatinine of 1.7, and has had stable kidney function with a follow-up of six months.

Case 2. A 20-year-old man was on hemodialysis for six months for chronic renal failure secondary to hypertension. The patient received a cadaveric graft that had been preserved with cold pulsatile perfusion for 22 hr at the time of transplant. At surgery, a subcapsular hematoma was evacuated.

Postoperatively, the patient required hemodialysis for ATN. On the sixth day, the patient developed a painful, tender mass over the graft, and the hematocrit fell from 15% to 12%. The patient was observed and transfused, and the coagulation abnormalities were corrected. On the eighth day, because of continued bleeding and enlargement of the mass, the graft site was explored. Two ruptures were identified on the convex surface of a markedly enlarged kidney. The graft was repaired by separate suturing of the ruptures and by wrapping in Gelfoam; 150–200 cc of blood was evacuated. Two biopsies were performed, one at the rupture site and the second at an uninvolved site. The biopsies showed ATN, mild cellular rejection, and vascular rejection. The rupture site was hemorrhagic and necrotic.

After repair, the patient remained oliguric and was treated with steroids and antithymocyte globulin (ATG). On the eighth day after repair, the patient began to bleed from a duodenal ulcer, which necessitated discontinuation of steroids and ATG. Because of continued gastrointestinal bleeding, the patient underwent graft nephrectomy, vagotomy, pyloroplasty, and oversewing of the ulcer on the nineteenth day after transplant.

Examination of the nephrectomy specimen revealed moderate cellular and vascular rejection. The patient has subsequently returned to hemodialysis and is awaiting another transplant.

Case 3. A 28-year-old man with chronic renal failure of unknown etiology was on hemodialysis for two and a half years. The patient received a cadaveric graft, which had been machine-preserved for 36 hr at the time of transplantation. Diuresis started 2 hr posttransplantation; this was followed by oliguria and the need for hemodialysis.

On the fourth postoperative day, the patient developed a painful, tender mass at the graft site, and his hematocrit fell. At exploration, a rupture was found at the upper pole. The rupture was sutured and wrapped with Gelfoam, and 400 cc of blood was evacuated. The biopsy showed ATN and mild cellular rejection.

On the second day after repair, diuresis occurred and renal function improved. He was discharged with a creatinine of 2.0, and his renal function has remained stable to date, 24 months after transplantation.

Case 4. A 42-year-old man was on hemodialysis for three years for renal failure secondary to hypertension. The patient had a living-donor renal transplant, which ended with graft nephrectomy, and right native nephrectomy for hypernephroma 1 year prior to cadaveric transplant. The cadaveric graft had been machine-preserved for 38 hr.

Postoperatively, the patient required hemodialysis for ATN. An open biopsy performed on the eighteenth postoperative day demonstrated mild glomerular, vascular, and cellular rejection. The patient showed no improvement in renal function, and on the thirty-first day he developed pain and tenderness over the graft, hypotension, and a falling hematocrit.

The patient was taken to the operating room, and a ruptured graft was found. Graft nephrectomy was performed. Examination of the graft revealed infarct and moderate vascular, glomerular, and cellular rejection. The patient has returned to dialysis and is stable at this time.

Case 5. A 58-year-old man was on hemodialysis for three years for renal failure secondary to hypertension. The patient underwent cadaver-donor renal transplant of a kidney that was preserved on perfusion machine for 18 hr. Postoperatively, the patient required hemodialysis for ATN.

On the fifth postoperative day, the patient developed tenderness and pain over the graft, hypotension, falling hematocrit, and bleeding from the drain site. On exploration, 1,000 cc of blood was drained, and a rupture at the upper pole of the graft was seen. Hemostasis was achieved with sutures and Gelfoam. No biopsy was performed.

After repair, the patient remained anuric, and his course was complicated by pancreatitis with ascites and *Legionella pneumoniphila*. CAT scan demonstrated a perinephric mass consistent with hematoma. In view of the complications and the continued anuria, graft nephrectomy was performed on the forty-first posttransplant day. At nephrectomy, a large perinephric hematoma was found. Pathologic examination indicated

TABLE 1
Graft Rupture—Clinical Features

Patient	Preservation technique/duration	Graft function prior to rupture	Indications of graft rupture	Day of rupture	Postrepair course
1	Ice/24 hr	ATN	Pain ↓ hematocrit ↓ blood pressure	9	Diuresis on Day 7 Stable function at 6 months
2	Machine/22 hr	ATN	Pain ↓ hematocrit	6	Oliguria Bleeding duodenal ulcer Nephrectomy
3	Machine/36 hr	ATN	Pain ↓ hematocrit ↓ blood pressure	4	Diuresis on Day 2 Stable function at 24 months
4	Machine/38 hr	ATN	↓ Hematocrit ↓ blood pressure	31	Nephrectomy at time of rupture
5	Machine/18 hr	ATN	Pain ↓ hematocrit ↓ blood pressure Blood from drain site	5	Anuria Pancreatitis *Legionnella pneumoniphila* Nephrectomy

ATN, acute tubular necrosis.

ATN, with partial recovery and mild cellular and vascular rejection.

The patient subsequently returned to dialysis and is stable at this time.

Results

Five cases of rupture of transplanted kidneys occurred in a total of 82 kidney transplants performed in the course of four and a half years (Table 1).

All 5 cases of rupture were characterized by a preceding ATN ranging from 4 to 31 days. One patient underwent diuresis for 2 hr which was followed by ATN.

Biopsies are available on all 5 cases and are consistent with ATN and mild to moderate rejection. No case demonstrated severe rejection (Table 2).

Salvage was attempted in 4 cases by patching or suturing the rupture and wrapping the graft in Gelfoam. In 2 cases, salvage was possible and the patients have maintained stable kidney function. The remaining 2 patients required nephrectomy at a future date owing to unstable medical conditions, which necessitated the cessation of immunosuppressive therapy. In the fifth case, salvage was not attempted as the graft had exhibited no function for 31 days, and renal

TABLE 2
Graft Rupture—Morphologic Features

	Type of specimen	Time posttransplantation	Glomeruli	Vascular rejection	ATN	Cellular rejection	Interstitial edema
1	Biopsy	1 hr	0	0	+	0	+1
	Biopsy	9 days	0	+1	+	+1–2	+2
2	Biopsy	8 days	Few PMN*	+1	+	+1	+1
	Nephrectomy (347 gm)	19 days	0	+1–2	+	+2	+2
3	Biopsy	4 days	Wrinkle GMB* +1	0	+	+1	+1
4	Biopsy	18 days	Transplant Glomerulopathy +1	+1	+	+2	+2
	Nephrectomy (340 gm)	31 days	Transplant Glomerulopathy +1	+2	+	+2	+2
5	Nephrectomy (205 gm)	41 days	Wrinkle GBM* +1	+1	+	+1–2	+1

* ATN, acute tubular necrosis; PMN, polymorphonuclear leukocytes; GBM, glomerular basement membrane.
Scale: 0 = none; +1 = mild; +2 = moderate; +3 = severe.

scans were consistent with very poor flow and function.

Discussion

Allograft rupture is not a rare event, and awareness of this serious complication combined with prompt surgical intervention can result in kidney salvage. The reported incidence of rupture ranges from 0.3% to 8.5%, according to Ghose, Oesterwitz, and Lord (1–3). It usually occurs within the first month posttransplant, but Lord et al have reported rupture even at 170 days (4). Many factors have been implicated in the etiology of graft rupture. These include rejection, ischemia, vascular damage, perfusion injury, previous biopsy, and trauma. The one constant factor in all these is graft swelling. Graft rupture in association with acute rejection was identified in the early cases of graft rupture by Lord and Haimov (4, 5). Rejection has been the most frequently implicated etiology of rupture (6–12). Although histologic studies of many of the reported ruptures demonstrated rejection, most were of mild to moderate intensity.

In our 5 cases, biopsies were obtained indicating mild to moderate rejection. No biopsy indicated severe rejection. All biopsies demonstrated ATN. Interstitial edema may be the response of the transplant kidney, stripped of its lymphatic drainage, to ATN and rejection. Edema was demonstrated in all the biopsies and appears to be a common factor in ruptures. In agreement with previous writers, including Dryburgh and Prompt, we have found no relationship between graft rupture and method of preservation or age of kidney at the time of transplantation (13, 14). We have attempted repair of 4 out of 5 ruptured grafts and have salvaged 2. Late nephrectomy was necessitated by medical complications in the other 2 patients. Careful monitoring of these patients following repair was required to guard against rebleeding.

In the presence of a ruptured graft, prompt surgical intervention should be undertaken. We have found inspection to be most useful in assessing the feasibility of graft salvage. The appearance of the kidney in terms of color, degree of swelling, size and number of ruptures, friability, and blood flow as indicated by bleeding are important hallmarks. We recommended intraoperative biopsy unless contraindicated by uncontrolled bleeding. Repair should be undertaken if the patient is stable, the kidney has a good macroscopic aspect, and the biopsy does not indicate irreversible rejection or necrosis.

Summary

Of 82 renal transplants performed at The Mount Sinai Hospital between January 1979 and June 1983, 5 cases of ruptured grafts were identified. All ruptures were preceded by a period of prolonged, acute tubular necrosis (ATN). All grafts were explored and biopsied, and graft salvage was attempted in 4 cases.

Intraoperative biopsies, as well as nephrectomy specimens, demonstrated ATN and mild to moderate rejection. Two patients have long-term renal function to date. Based on our results, we recommend prompt graft exploration, intraoperative biopsy, and repair of rupture if the patient is stable and the graft appears salvageable both microscopically and macroscopically.

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An Antireflux Modification in Ureterosigmoidostomy

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Abstract

Ureterosigmoidostomy is among the oldest forms of continent urinary diversion. Many variations and modifications in technique have been reported over the years. A new method of ureterosigmoidostomy is described. It has been applied clinically in a series of eight patients with satisfactory results. A revival of this once popular procedure cannot be expected. However, there are definite indications for its use in a selected group of patients.

Ureterosigmoidostomy is one of the oldest forms of urinary diversion. Its most common complications are ascending infection causing pyelonephritis, and the metabolic disturbances, mainly hyperchloremic acidosis and hypokalemia. Because of its complications, the operation fell into disrepute and was supplanted mainly by ureteroileocutaneous anastomosis (ileal conduit, Bricker's operation) (1, 2).

However, in carefully selected patients, ureterosigmoidostomy is well tolerated. Urinary continence and the absence of a stoma or an external appliance are appealing features. The operating time is shorter than in other diversionary procedures.

I report here on a new technique applied clinically in eight patients with satisfactory results.

Patients and Methods. The eight patients selected (Table 1) had a life expectancy of not over 20 years. A prerequisite was the presence of a normal colon and good anal sphincteric tone. Most of the patients had rejected a priori the idea of wearing an external appliance or of having a stoma.

The patients underwent a standard bowel preparation and were operated on under general anesthesia. Postoperatively the patients had intravenous urograms every 6 months and barium enemas as necessary.

Patient No. 7 had a preexisting sigmoid colostomy for inoperable colorectal cancer. A vesicorectal fistula formed, necessitating supravesical diversion. An additional stoma was refused by the patient, and a ureterosigmoidostomy transplanting the ureters into the sigmoid loop leading to the colostomy was performed, thus creating a "wet colostomy." Although the conditions existing in a ureterosigmoidostomy in continuity do not fully apply, the patient is included in this series because of the new technique used.

Technique. The technique employed was arrived at by combining some of the principles of Goodwin's open transcolonic ureterointestinal anastomosis (3), which affords excellent exposure, and Paquin's method of ureteroneocystostomy (4).

A left paramedian incision extending from symphysis pubis to umbilicus (Fig. 1A) is used. The peritoneal cavity is entered and explored. The liver, spleen, and all other viscera are inspected. Cystectomy, if indicated, is performed first.

The left ureter is exposed laterally to the sigmoid colon, mobilizing the sigmoid and its mesentery medially. The right ureter is exposed by incising the posterior peritoneum above the pelvic brim. The sigmoid colon is "fixed" to the posterior peritoneum below the area of the sacral

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TABLE 1
Clinical Characteristics of Eight Patients

Pt. no. & date of operation	Age & sex	Diagnosis	Grade & stage	Preop therapy radiol or chemo
1 6/23/75	65 M	TCC Bladder CIS	I-II A	5000 RADS
2 4/30/79	83 M	TCC bladder	II-III B_1	6500 RADS
3 2/19/82	75 M	Bladder squamous cell ca	II-III	6600 Rads
		TCC	B_2-C	Syst. Adriamycin, 5 FU, Mitomycin
		Prostate: adenoca	A_1	Intravesical
4 11/18/82	63 F	TCC bladder	III-IV B_2-C	None
5 6/2/83	81 F	TCC bladder	III-IV B_1	None
6 8/11/83	76 M	TCC bladder	III C	None
7 12/15/83	62 M	Ca rectum		Cobalt
		Rectovesical fistula		Chemotherapy
8 11/30/84	73 M	TCC bladder	III B_1	
		Adenoca prostate	A_2	None

promontory using on each side a 2:0 silk suture (Fig. 1B). The ureters are placed on each side of the sigmoid, which is opened at its anterior surface for a distance of 5.0 cm. Through this open colotomy the ureters are brought in separately on each side of the colon. A formal tunnel is not attempted, although an oblique course is followed.

Each ureter is brought in obliquely through a small opening made with a curved hemostat placed from the inside of the bowel lumen. A row of 3:0 silk sutures is placed between the outer wall of the ureter and the bowel serosa to slightly telescope the ureter and thus reduce tension. The ureter is prepared (Fig. 1C) by excising any redundant segment and spatulated. A cuff about 1.0 cm high is prepared in the standard fashion. After the cuff is fully completed, it is sutured to the adjacent colonic mucosa. In the future we plan to prepare the cuff by fishmouthing the ureter. The ureter is stented with a No. 8F Silastic tubing brought out through the lumen of a rectal tube, which has been placed at the beginning of the operation and secured to the perianal area with a silk suture. A similar procedure is done on the contralateral side (Fig. 2). The colotomy is closed in two layers, a first row of 2:0 interrupted chromic catgut and a second one of 2:0 interrupted silk. The incision is closed in routine fashion. The rectal tube and ureteral stents are kept in place for about 7–10 days. At that time they are usually expelled spontaneously. A postoperative intravenous urogram is obtained thereafter, as well as a barium or gastrografin enema.

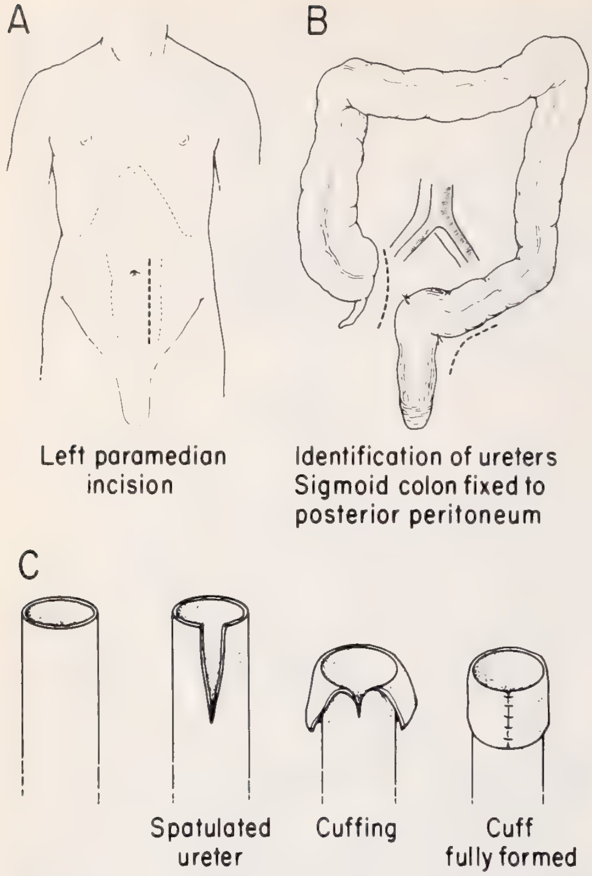
Results. The postoperative results—course and survival, presence of ascending infection and pyelonephritis, pre- and postoperative findings on intravenous urogram, as well as surgical interventions, such as anastomotic revisions and reoperation related to the original surgery—have been good (Table 2).

A representative preoperative urogram (patient 6) is within normal limits, and the postoperative intravenous urogram of the same patient shows normal upper tracts (Fig. 3). A gastrografin enema demonstrates absence of coloureteral reflux (Fig. 4).

The overall results were generally gratifying and satisfactory. Only one patient expressed dissatisfaction with the procedure and could not adjust psychologically to it. In no instance was the ureterosigmoidostomy converted to a different form of diversion.

Historical Review. Urinary diversion to the lower bowel was introduced in the latter part of the nineteenth century in cases of bladder exstrophy. Since then as many as 75–80 variations in technique have been described. Simon (5) in 1852 was the first to report on ureterosigmoidostomy. Maydl (6) (1894) transplanted the trigone into the lower bowel with the ureterovesical junction intact. Bergenhem (7) (1895) transplanted the ureters separately into the rectum extraperitoneally. Peters (8) (1899) used a similar technique. The operation of ureterosigmoidostomy was then commonly referred to as Peters' operation.

Coffey (9, 10) (1911) popularized uterosigmoidostomy, introducing a valvelike principle at the ureterocolonic junction designed to prevent reflux. Subsequently, Coffey described three other methods, but the Coffey I technique remained the standard procedure for years. Cordonnier (11) (1949) introduced an end-to-side, mucosa-to-mucosa anastomosis. Nesbit (12, 13) (1949) described an anastomosis by direct elliptical mucosa to mucosa connection. Leadbetter


A
B
Left paramedian incision
Identification of ureters Sigmoid colon fixed to posterior peritoneum
C
Spatulated ureter
Cuffing
Cuff fully formed

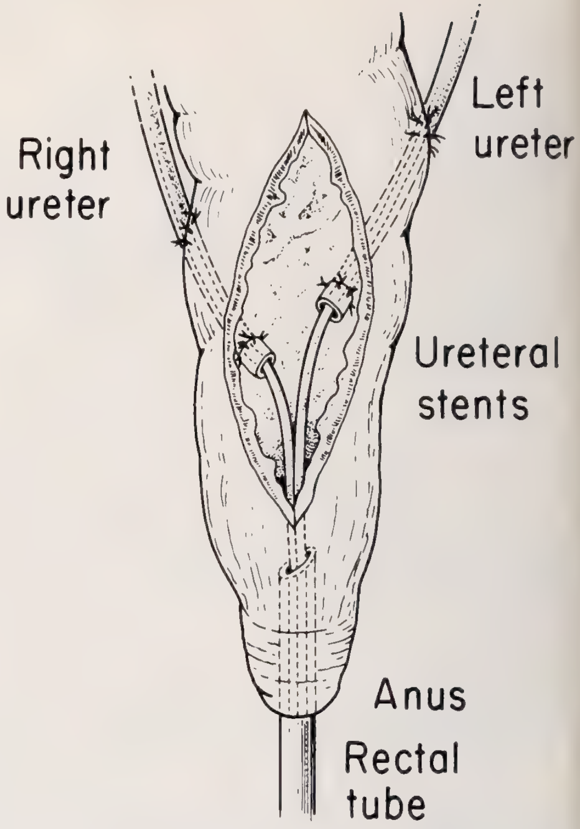

Right ureter
Left ureter
Ureteral stents
Anus
Rectal tube

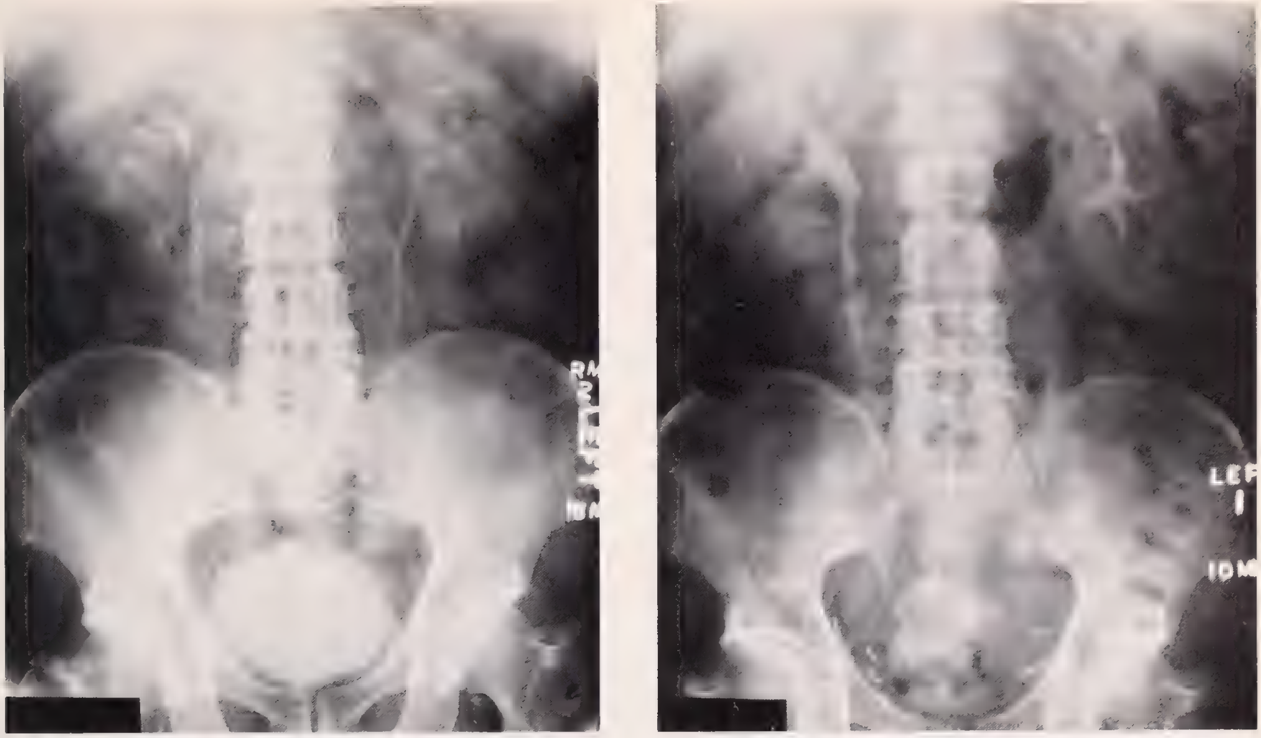
Fig. 1. (**A**) Left paramedian incision used for ureterosigmoidostomy. (**B**) Identification of ureters and preparation of sigmoid colon fixed to posterior peritoneum. (**C**) Preparation of ureter for ureterosigmoid anastomosis.
Fig. 2. Ureterosigmoid anastomosis completed as seen through open colotomy.

(14) (1951) used a combination of mucosa-to-mucosa principle in addition to a short submucosal tunnel to prevent reflux and stenosis. Mathiesen (15) (1953) created a nipple anastomosis, a kind of intracolonic nipple or papilla, by wrapping a flap of whole thickness of the bowel wall around the terminal 2.0 cm of ureter.

Goodwin (3, 16, 17), a major proponent of

TABLE 2
Postoperative Results in Eight Patients

Pt. no.	Preop IV urogram	Postop IV urogram	Degree of metabolic acidosis	Revision or reoperation	Clinical course and survival
1	Contracted bladder, massive bilateral hydronephrosis	Improved upper tracts	Moderate	Left nephroureterectomy for TCC Ureteropelvic junction 10-11-84	Free of disease 10 years
2	Contracted bladder, massive bilateral hydronephrosis	Improved upper tracts	Moderate	Intestinal obstruction 10-25-79	Free of disease 6 years
3	Normal	Unchanged	None	Intestinal obstruction 1984	Free of disease 3 years
4	Nonvisualizing right kidney	Improved visualization of small right kidney	Mild	None	Free of disease 2+ years
5	Nonvisualizing left kidney	Unchanged	Mild	None	Lost to follow-up
6	Normal	Unchanged	Mild to moderate	None	Died of metastatic disease 9/11/84
7	Normal	Unchanged	None	None	Alive with disease
8	Normal	Unchanged	None	None	Alive, free of disease



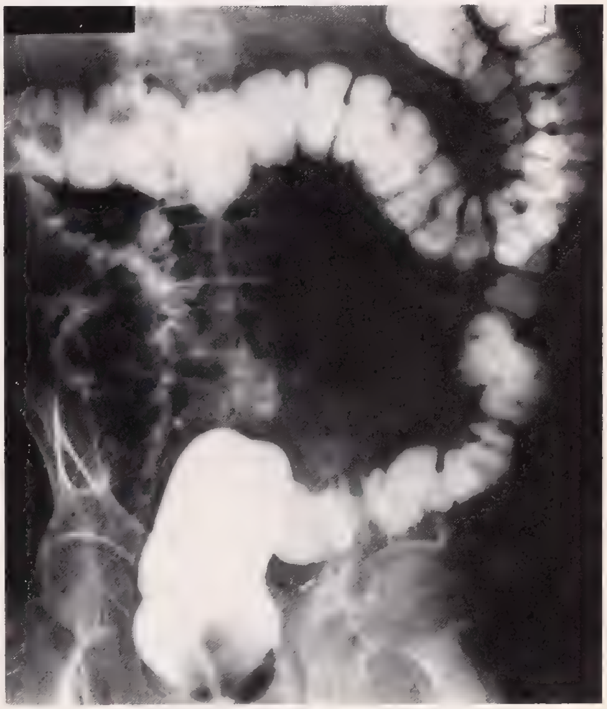


Fig. 3. Above left Preoperative IVU of patient No. 6 is essentially within normal limits. **Above right** Same patient's postoperative study shows normal upper tracts and filling of sigmoid colon.
Fig. 4. Right Gastrografin enema of same patient demonstrates absence of colouretereral reflux.

ureterosigmoidostomy, introduced in 1953 his technique of open transcolonic ureterointestinal anastomosis with tunnel and direct mucosal connection.

Discussion. With all the improvements, variations, modifications, and refinements in technique, ureterosigmoidostomy has been plagued by many complications (17–19).

Hyperchloremic acidosis with hypokalemia (17–23) secondary to ureterosigmoidostomy is a tubular disorder. Tubular damage is caused by stasis and infection (pyelonephritis). There is, in addition, active reabsorption of urinary chloride by the intestinal mucosa with only slight reabsorption of potassium and sodium. The disorder is corrected by giving sodium bicarbonate and potassium citrate, keeping the patients on a low-chloride diet, and instructing them to empty their bowel every 2–3 hr.

Ascending infection and pyelonephritis were common complications before the introduction of antireflux techniques in ureterosigmoidostomy, but even today the problems of late stenosis and obstruction have not been solved.

The literature is full of reports on the development of late colonic carcinoma (24, 25) close to the sites of ureteral anastomosis in the sigmoid colon. This is usually a late occurrence, often after 20–40 years, and therefore of no consequence in patients with a life expectancy of less than 20–25 years. The situation is altogether different in children.

If the ureterosigmoidostomy is not well tolerated, a proximal diverting colostomy will solve the problem by separating the fecal and urinary streams and leaving the patient with a rectal bladder, which most patients can deal with more easily.

The main advantage of the presently described antireflux modification is prevention of refluxing feces up the ureters with the known and serious sequelae of chronic pyelonephritis and, ultimately, renal failure. The limited number of cases precludes any long-term statistical projections.

Ureterosigmoidostomy is certainly not the ideal form of urinary diversion, which has yet to be found. However, ureterosigmoidostomy is relatively well tolerated in a selected group of patients. The absence of a stoma and of a collecting device, as well as satisfactory continence, make it appealing. It deserves a place in every urologist's armamentarium.

AUTHOR'S NOTE: Since the acceptance of this paper one more patient was added to the series. Patient no. 2 died at age 90 of unrelated causes.

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Pancreatoduodenectomy Preserving a Functioning Pylorus, the Stomach, and a Pancreatic Remnant: A Complex Functional Evaluation

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Abstract

A new method was developed for partial pancreatoduodenectomy in patients with chronic pancreatitis that preserves the pylorus and stomach, the pancreatic remnant being either occluded or anastomosed to the antrum. Gastric and pancreatic function, intestinal absorption, and the findings of upper-gastrointestinal endoscopy and histologic studies were evaluated about one year after the operation. In patients with the occluded remnant, the pancreatic function tests, steatorrhoea, and vitamin B_{12} absorption were highly abnormal, and diabetes frequently developed. In patients with pancreaticogastrostomy, these functions were closer to normal and endocrine function was preserved. In these patients, unlike patients with Whipple's operation, the pancreatic secretory pattern, gastric emptying, and gastrin secretion and release were normal. Gastritis, jejunitis, and cholangitis occurred less frequently. Pancreatic function did not deteriorate during the observation period. We conclude that the preserved gastric reservoir, with a functioning pancreatic remnant stimulated by virtually intact gastrointestinal neurohormonal mechanisms, may be advantageous for patients requiring proximal resection of the pancreas.

Partial pancreatoduodenectomy is indicated for unrelenting pain in chronic pancreatitis whenever the lesions are localized or more advanced in the head of the pancreas. The results of Whipple's operation are unsatisfactory because of the loss of gastric reservoir function, the development of dumping and blind-loop syndromes, as well as ineffective stimulation of the pancreatic remnant. A new method, preserving the pylorus and stomach (1, 2), with the pancreatic remnant anastomosed to the antrum (3, 4) or occluded with Ethibloc or other glues (3, 5), was recently developed for this type of proximal resection in chronic pancreatitis (Fig. 1).

When the pancreatic duct is occluded, exocrine secretion of the residual pancreas is of course lost. On the other hand, an anastomosis between the pancreatic duct and the gastrointestinal tract is unnecessary. With a pancreaticogastrostomy, however, exocrine function of the remnant, stimulated by the almost intact neurohormonal mechanisms, is preserved.

The aim of this study was to assess and compare function after both types of partial pancreatoduodenectomies preserving the pylorus and stomach in order to determine which is the most physiologic procedure.

Patients and Methods. Thirty-four patients (3 women and 31 men) with severe chronic alcoholic pancreatitis were studied after partial pancreatoduodenectomy. For 13 patients, a classical Whipple's procedure was performed; in 12 pa-

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TABLE 1
Clinical Data by Type of Partial Pancreatoduodenectomy

		Preserved pylorus	
	Whipple's operation	with pancreatico-gastrostomy	with occluded pancreatic duct
N	13	12	9
Age	38 ± 2.5	47 ± 1.0	44 ± 1.4
p	NS	NS	—
Months postop	13 ± 4.0	8 ± 1.7	13 ± 3.1
p	NS	NS	—
Kay test*			
BAO (mEq/h)	—	1.0 ± 0.5	0.1 ± 0.05
range	0–2.7	—	0.04
p	NS	NS	
cMAO	—	11.8 ± 2.8	10.4 ± 21
range	0–2.71	—	0.9–21
p	NS	NS	—
Gastritis	Regularly	5/12	4/8
Jejunitis	Regularly	7/12	3/8
γGT (U/l)	289 ± 133	35 ± 7	107 ± 39
p	<0.05	<0.05	—
AP (U/l)	86 ± 25	42 ± 4	75 ± 13
p	<0.05	<0.01	—
Xylose loading			
>lg/5hr	—	1.7 ± 0.15	1.7 ± 2.0
Schilling test			
>10%	—	14.9 ± 2.58	7.2 ± 0.91
Manifest diabetes			
preop	1/13	0/12	2/9
postop	4/13	0/12	6/9
Reactive hypoglycemia	3/9	1/12	0/3
Calcification			
preop	2/13	1/12	0/9
postop	1/13	4/12	3/9

* Not performed after Whipple's operation, for technical reasons.

tients the pylorus was preserved with pancreaticogastrostomy; in 9 patients, pyloric preservation without anastomosis, but with occlusion of the pancreatic duct had been performed several months previously.

Gastric function, intestinal absorption, and pancreatic function were studied in all patients. Endoscopy and histological analyses were done. Gastric emptying was examined with Tc-S-colloid labeling of a mixed meal (2). Gastrin secretion was estimated after a mixed meal with radioimmunoassay, using a C-terminal specific antibody. The Kay test for gastric acid secretion (6), a xylose loading test (7), the Schilling test (8), a test for fat in feces (9), an iron loading test (10), and tests of gamma glutamyl transpeptidase (11), alkaline phosphatase (12), starch tolerance (13), and Lipiodal absorption (14) were all performed according to the methods originally described. Glucose loading with 100 gm glucose was done in patients without manifest diabetes mellitus. Pancreatic secretion was measured by the secretin-pancreozymin test (15) and the Lundh test (16).

Statistical evaluation of test results was by Student's *t* test for unpaired values, using $p < 0.05$ as determining statistical significance.

Results. There were no significant differences in patient age or length of time elapsed between operations and testing (Table 1). The Kay test was not performed after Whipple's procedures for technical reasons. In patients without gastric resection, mean basal and stimulated acid secretion was normal; hypoacid and hyperacid conditions were distributed about equally. Endoscopy demonstrated no jejunal ulceration in any patients. Histologic findings regularly confirmed gastritis and jejunitis after Whipple's operation, but these were less frequent when the pylorus was preserved. Gastrin release (Fig. 2) as well as gastric emptying (Fig. 3) was normal in patients without gastric resection, but not after Whipple's procedure.

In plasma, cholestatic enzymes such as gamma glutamyl transpeptidase and alkaline phosphatase were frequently elevated after Whipple's operation as well as in the patients with preserved pylorus and occluded pancreatic duct, but were not elevated after pancreaticogastrostomy (Table 1).

Schilling tests demonstrated impaired vitamin B_{12} absorption in all patients with a preserved pylorus without pancreatic anastomosis, but only in one patient among those with pancreaticogastrostomy. Xylose loading was normal in both groups (Table 1). Iron loading resulted in significantly lower levels of blood iron at the later phases of absorption in patients with duct occlusion and Whipple's operation than in patients with pancreaticogastrostomy (Fig. 4).

Pancreatic secretion did not decrease during the late postoperative period in patients with pancreaticogastrostomy; this was shown in repeated examinations by the secretin-pancreozymin and Lundh tests. In fact, the volume even increased, demonstrating stable exocrine function and a patent outlet from the pancreatic remnant (Fig. 5). In patients with a preserved pylorus and pancreaticogastrostomy, the secretory pattern of the pancreas, although at low levels, was normal (Fig. 6): the Lundh test meal resulted in larger enzyme responses than did exogenous stimulation with synthetic secretin or cholecystokinin octapeptide. After Whipple's operation, the test meal was close-to-ineffective for trypsin and lipase secretion, but not for amylase output. In patients with Whipple's operation, compared to those patients with the pylorus preserved and a pancreaticogastrostomy, the amylase-dependent

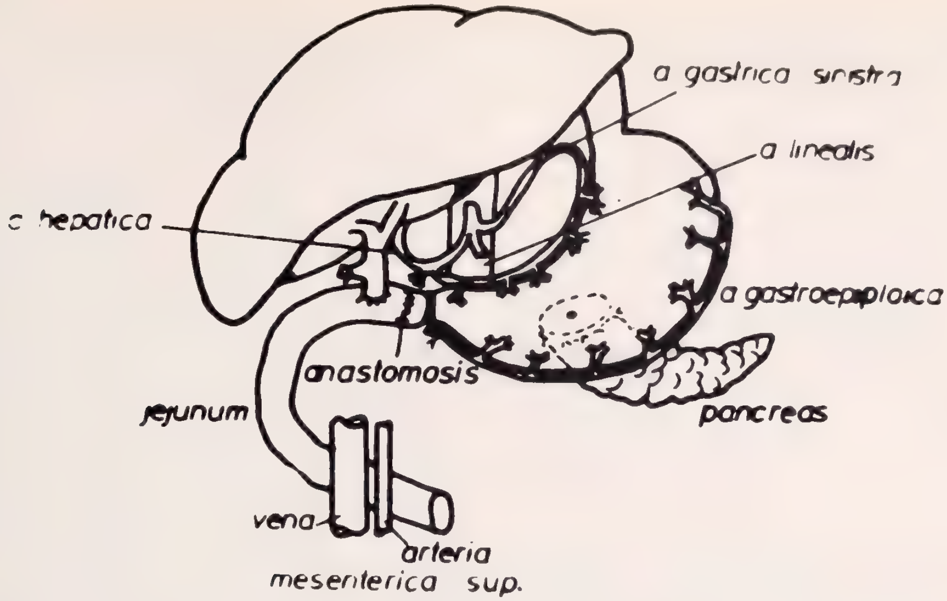


Fig. 1. Surgical technique of partial pancreatoduodenectomy with preserved pylorus and pancreaticogastrostomy.

starch tolerance test yielded less abnormal results, but the lipase-dependent Lipiodol test, performed with the liquid Lundh test-meal, showed more abnormal results. (Table 2). The results of fat-loading using mixed meals were equal in both groups. All these indirect pancreatic-function tests naturally yielded highly abnormal results after duct occlusion.

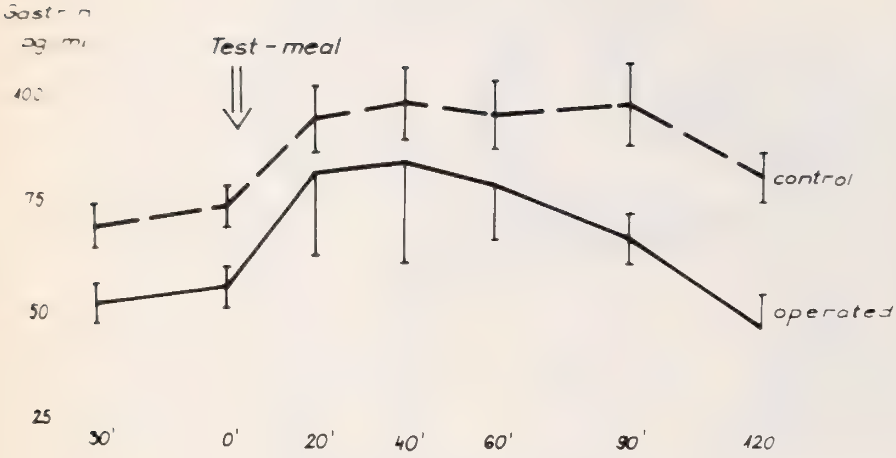
Glucose tolerance, which was examined in patients without manifest diabetes, was decreased in all patients with occluded pancreatic duct, but in fewer patients after pancreaticogastrostomy or Whipple's operation (Fig. 7).

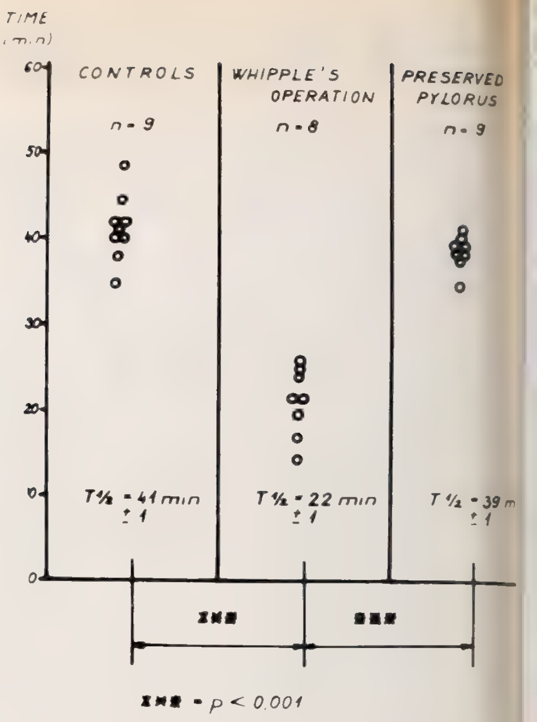
Manifest diabetes occurred with a fairly similar frequency in all the groups, but its incidence did seem to increase after Whipple's procedure. Pronounced reactive hypoglycemia (3 mM/L or less), linked to late dumping syndrome, was rather frequent in Whipple's-operation patients (Table 1). Calcification of the pancreas occurred after the operations preserving the pylorus, with and without pancreaticogastrostomy, but not after Whipple's operation; in one case it persisted (Table 1). A return to alcohol consumption was observed in most patients with postoperative calcification.

Discussion. The late postoperative results of Whipple's operation are variable because of loss of gastric reservoir function, dumping, and blind-loop syndrome (1), and also because of ineffective stimulation of the pancreatic remnant. This results from the failure of upper gastrointestinal neurohormonal mechanisms, such as antro-pyloro-duodeno-pancreatic reflexes (17, 18) releasing gastrin, cholecystokinin, secretin, and other hormones (19). Indeed, gastric emptying was significantly disturbed and reactive hypoglycemia frequently occurred in our post-Whipple patients demonstrating dumping syndrome. Accelerated gastric emptying of liquid meals, but not of solid ones (20), may partially explain the finding that the fat absorption test, using the liquid Lundh test-meal, yielded more abnormal results than the fat-loss test, using a mixed meal. Blind-loop syndrome and mild cholangitis (21) may explain the frequently elevated cholestatic enzymes after Whipple's operation. In patients without gastric resection but with a preserved pylorus, gastric secretion, gastrin release, and gastric emptying were normal, and no jejunal ulceration was observed. A functioning pylorus is apparently sufficient for prevention of jejunal ulcers even in hyperacid patients (1), possibly because of intact feedback regulation of gastrin release (22).

Gastritis and jejunitis did not occur more frequently after pancreaticogastrostomy than without it, indicating that pancreatic juice without bile is not harmful to the gastric mucosa. On the contrary, gastritis regularly occurred after Whipple's operation because of biliary reflux.

Bacterial overgrowth in consequence of maldigestion (21) and absence of the antibacterial ef-





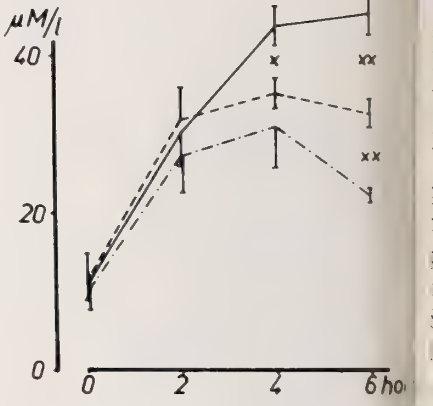
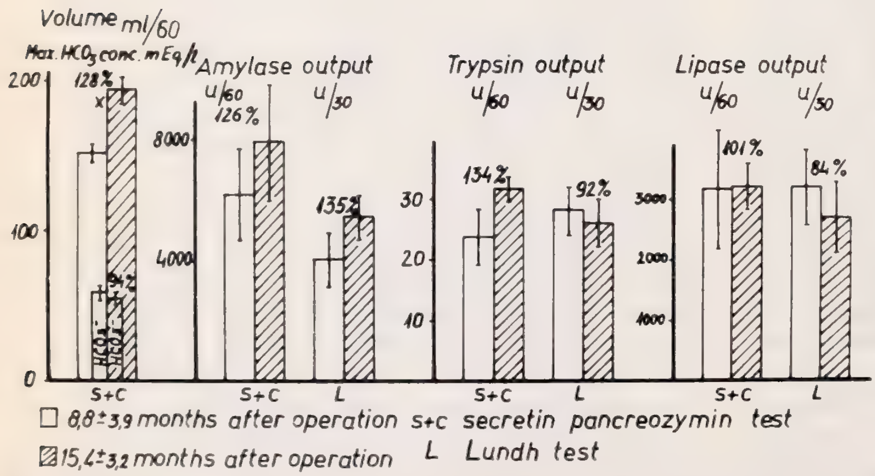


Fig. 2. **Above left** Gastrin responses to mixed meal in patients with partial pancreatoduodenectomy with preserved pylorus (n = 4) and in controls (n = 7). Values are mean ± SE.

Fig. 3. **Above right** Gastric emptying measured with Tc-S-colloid labeled semiliquid meal after partial pancreatoduodenectomy with and without gastrectomy. Individual values and means ± SE of half-times (T ½) and significant differences ($p < 0.001$) are indicated.

Fig. 4. **Right** Iron levels after intake of 1 gm of ferrum reductum in patients with partial pancreatoduodenectomy; (—), preserved pylorus and pancreaticogastrostomy (n = 12); (---), preserved pylorus and duct occlusion (n = 9); (- · -), Whipple's operation (n = 4). Values are mean ± SE. Significant differences are indicated either as X($p < 0.05$) or XX($p < 0.01$).



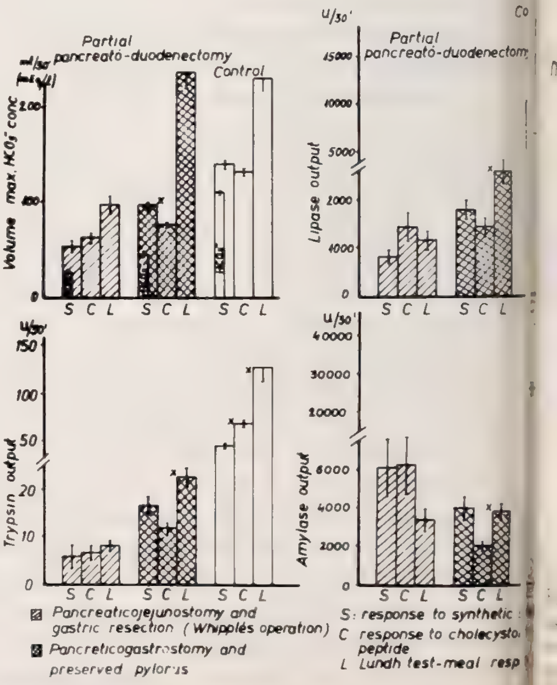


Fig. 5. **Above left** Pancreatic secretory pattern after pancreatoduodenectomy. Values are mean ± SE.

Fig. 6. **Above right** Late changes of pancreatic secretion in patients repeatedly examined after operation. Values are mean ± SE of 5 pat Significant increase in volume is indicated by X($p < 0.05$).

fect of pancreatic juice (23) may explain the bacterial contamination of bile that resulted in elevated cholestatic enzyme levels in patients with pancreatic duct occlusion. Similar factors may account for the changes in iron absorption and vitamin B_{12} absorption.

Pancreatic proteases are needed to split salivary and gastric R protein from the vitamin B_{12}-intrinsic-factor complex before absorption (24, 25). Indeed, the abnormality of values in the Schilling tests was decreased by pancreatic supplements. The equally normal xylose absorption in both groups excludes a major role for intestinal factors.

In contrast to the results of Whipple's operation, in patients with a preserved pylorus and pancreaticogastrostomy, the exocrine secretory patterns of the remnant, stimulated by the intact antro-pyloro-pancreatic reflexes, and by the release of gastrin, upper jejunal cholecystokinin, secretin, gastric inhibitory peptide, and other hormones (19), were similar to those of the controls, despite the loss of functional capacity due to chronic pancreatitis and parenchymal resection. The preserved neurohormonal regulation of the pancreatic remnant and the normal gastric emptying may explain the Lipiodol test findings. The patients' subjective evaluations after the new operation were superior to those after Whipple's. Stable amylase (26) accumulating in the afferent loop during the basal phase because of adaptation regulated by insulin-glucose mechanisms (27) may explain the diversity of starch tolerance between the two operative methods.

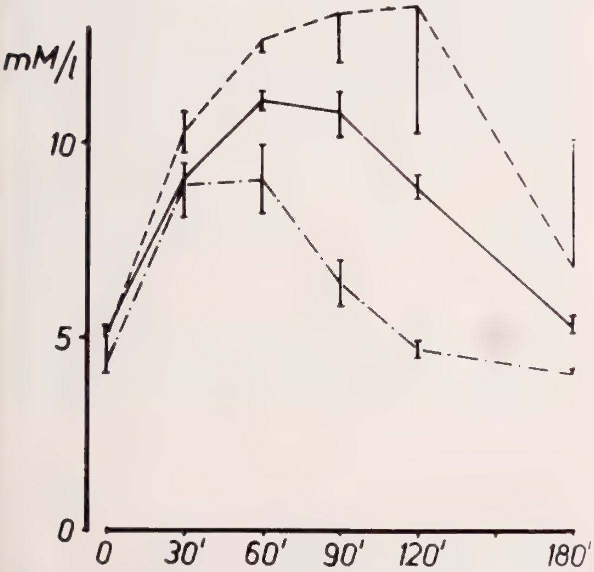


Fig. 7. Blood glucose levels after loading with 100 gm of glucose in patients with partial pancreatoduodenectomy; (---), preserved pylorus and duct occlusion (N = 3); (—), preserved pylorus and pancreaticogastrostomy (N = 12); (- · -), Whipple's operation (N = 9).

TABLE 2

Results of Indirect Pancreatic-Function Tests by Type of Partial Pancreatoduodenectomy

	Whipple's operation	Preserved pylorus with pancreatico-gastrostomy	Preserved pylorus with occluded pancreatic duct
Starch tolerance*	211 ± 28.9	708 ± 131.9	1525.0 ± 225.0
p	<0.05	<0.001	—
Lipiodol†	0.8 ± 0.20	2.3 ± 0.65	0.6 ± 0.17
p	<0.05	<0.01	—
Fat load‡	35.4 ± 7.50	36.2 ± 6.05	90.3 ± 9.45
p	NS	<0.001	—

* Limit of normal values <70%.
† Limit of normal values >2.8.
‡ Limit of normal values <7%.

The frequency of manifest and latent diabetes increased after duct occlusion, in contrast to results in the group with pancreaticogastrostomy. This is in accordance with our previous finding (28), but in contrast to the results of others (5). Progressive sclerosis and the loss of insular cell mass may explain the decreased endocrine function after pancreatic resection combined with duct occlusion. Regeneration of islets may occur in patients with preserved pylorus and pancreaticogastrostomy.

Calcification of the pancreatic remnant progresses in patients without gastric resection as a result of continued alcoholic consumption. Preserved gastrin release (29) and cholinergic reflexes (30) may explain the increased susceptibility of pancreas to alcohol in these patients. Finally, neither endocrine nor exocrine function progressively diminished during the late postoperative period in our patients with pancreaticogastrostomy.

In conclusion, preservation of the pylorus and anastomosis of the pancreatic remnant to the antrum after resection of the pancreatic head appear to be advantageous for digestion and quality of life. Patients benefit from the remaining exocrine and endocrine function stimulated by gastrointestinal neurohormonal mechanisms, as well as the intact gastric reservoir.

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Improved Results in Resection of Abdominal Aortic Aneurysms, 1963–1984

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Abstract

To determine how operative risk in resection of abdominal aortic aneurysms has changed, we reviewed the records of 429 patients we operated on from 1963 through 1984 for intact infrarenal abdominal aortic aneurysm. We found that operative mortality declined from 8% (1963–1974) to 2% (1975–1984), which is less than the risk of rupture of all but the smallest aneurysms. The improved results are attributed to better diagnosis and treatment of heart disease, woven Dacron grafts, a durable monofilament suture for anastomosis, avoidance of bank blood, and an experienced surgical team. The authors advise resection of aneurysms of 5 cm or greater diameter and of many smaller ones. All patients with nonresected aneurysms must be followed up; enlargement demands early surgical intervention. Older patients and patients with other risk factors should not be excluded summarily from aneurysmectomy but must be individually evaluated, recognizing the poor prognosis once rupture occurs.

During the past 20 years there have been advancements in the management of patients undergoing elective resection of an abdominal aortic aneurysm which should lessen the risk of operation. The operative risk has been reported at 1.7% to 8% (1–6); one group has reported 0.9% (7) in a selected group of patients. Because the decision to recommend surgery, especially for small aneurysms and for patients with other risk factors, is influenced by the safety of the operation, we thought it important to determine the operative risk at present. To do so, we reviewed the records of patients we operated on for an intact abdominal aortic aneurysm and compared the results from the earlier years (1963–1974) with those from the later years (1975–1984).

Clinical Characteristics of Patient Population

From 1963 through 1984, 429 patients underwent resection of an intact infrarenal abdominal aortic aneurysm, including one patient with an isolated common iliac artery aneurysm. From 1963 through 1974, 144 operations were performed; there were 285 from 1975 through 1984. Of the total, 353 patients were male and 76 were female. Of those operated on in the earlier period, 47% were 70 years of age or older (mean age, 69 years); in the later period, 54% were 70 years of age or older (mean age, 70 years) (Table 1).

Two hundred ninety-four patients (69%) had one or more risk factors (Table 2). The incidence of risk factors may be higher than that listed because some records did not include information about a risk factor and if not included we assumed the disease was not present.

All aneurysms were resected and aortic continuity was restored with a woven Dacron graft (the material currently in use) in 398 patients and with a knitted Dacron graft (in use initially) in 31 patients (Table 3). Suture material used for anastomosis (Table 4) included Polyethylene during the earlier years (discarded because of late deterioration) and polypropylene, which is now used almost exclusively. Of the associated

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TABLE 1
Age of Patients

Age (years)	1963–1974 (*n* = 144) No.	1975–1984 (*n* = 285) No.	Total (*n* = 429) No.
<49	3	4	7
50–59	9	25	34
60–69	65	102	167
70–79	59	115	174
80–89	8	36	44
>90	—	3	3
% 70 & up	47%	54%	
Mean, yrs	69	70	

TABLE 2
Risk Factors

	1963–1974 (*n* = 144), No. (%)	1975–1984 (*n* = 285), No. (%)	Total (*n* = 429), No. (%)
Cardiac*	59(41%)	99(35%)	158(37%)
Hypertension†	58(40%)	116(41%)	174(41%)
Diabetes†	10 (7%)	17 (6%)	27 (6%)
Lung disease‡	18(13%)	42(15%)	60(14%)
Renal§	6 (4%)	23 (8%)	29 (7%)
One or more	104(72%)	190(67%)	294(69%)

* Defined as history of myocardial infarction, angina, congestive heart failure, or cardiomyopathy. Excludes patients who had prior coronary artery bypass operation because their coronary disease had been repaired.

† Defined as requiring medication.

‡ Defined as under treatment or had significantly impaired pulmonary function on a pulmonary function test.

§ Defined as preoperative serum creatinine greater than 1.9 mg% (normal, 0.7 mg%–1.4 mg%).

TABLE 3
Type of Dacron Graft Material

	1963–1974	1975–1984	Total
Knitted	31	0	31
Woven	113	285	398
TOTAL	144	285	429

TABLE 4
Suture

	1963–1974	1975–1984	Total
Silicone polyester*	117	213	330
Polypropylene†	1	71	72
Polyethylene‡	19	0	19
Teflon polyester§	2	1	3
Polyester‖	4	0	4
Not listed	1	0	1
TOTAL	144	285	429

* Ticron, manufactured by Davis and Geck.

† Prolene, manufactured by Ethicon.

‡ Manufactured by Davis and Geck.

§ Tevdek, manufactured by Deknatel.

‖ Dacron, manufactured by Davis and Geck. Mersilene, manufactured by Ethicon.

operations done at the time of the aortic resection (Table 5), the 4 nephrectomies were planned, but the splenectomy was done because of a tear during resection of the aneurysm. Three patients had repair of a femoral artery because of injury during preoperative aortography.

Results

Mortality. For the entire series, 16 patients (3.8%) died within 30 days of operation or during continuous hospitalization (Table 6). Seven patients died of heart disease. The dye allergy which caused one death was activated during a postoperative computerized tomography scan ordered incidental to the patient's recovery. The multiple-system failure in one patient included renal failure, sepsis, ileus, and pulmonary insufficiency requiring tracheostomy. The patient whose death was caused by paraplegia died of ensuing complications 4 months after surgery but never left the hospital. The operative mortality in the early period was 8% (11 deaths/144 operations), and in the later period 2% (5 deaths/285 operations). If the patient who died 4 months postoperatively of paraplegia and the patient who died of dye allergy are excluded, the operative mortality for the entire series is 3.3%, with an operative mortality of 7% for the early period and 1.4% for the later period.

Complications. Cardiac complications were the most frequent, accounting for 44% of all complications (Table 7). Of the 11 patients who had a postoperative myocardial infarction, 5 were in the 1963–1975 group (4 deaths) and 6 were in the 1975–1984 period (1 death). Patients classified as having arrhythmia and congestive heart failure did not have a myocardial infarction. Although some patients had a transient rise in serum creatinine after operation, in the renal failure group we included only patients who required dialysis. Of these there were 5; 3 had a preoperative serum creatinine of greater than 2 mg%, 1 was the patient who died of multiple-system failure, and 1 had a nephrectomy at the time of aortic resection for a chronically occluded renal artery. The group of patients with emboli included patients with clot and atheroemboli (trash foot), and the 4 patients with this complication recovered without amputation. As noted previously, the splenectomy was caused by injury during the aortic operation. Of the 2 patients with paraplegia, 1 recovered completely and 1 was the patient who died. Patients with intestinal obstruction or postoperative hemorrhage were included only if they required reoperation.

Twenty-nine patients had undergone coronary artery bypass operation prior to aortic resection. All coronary operations were performed after 1974. Postoperatively, 1 of the 29 had an arythmia and survived, but none had a myocardial infarction or congestive heart failure.

TABLE 5
Associated Operations

	1963–1974 (*n* = 144)	1975–1984 (*n* = 285)	Total (*n* = 429)
Renal artery repair	4	15	19
Cholecystectomy	3	11	14
Femoral-popliteal graft	1	3	4
Nephrectomy	1	3	4
Hernia repair		4	4
Horseshoe kidney	1	1	2
Mesenteric artery		1	1
Coronary artery graft		1	1
Appendectomy		1	1
Vagotomy-pyloroplasty		1	1
Splenectomy		1	1
Femoral artery repair	1	2	3
TOTAL	11(8%)	44(15%)	55(13%)

TABLE 6
Cause of Death

	1963–1974 (*n* = 144)	1975–1984 (*n* = 285)	Total (*n* = 429)
Cardiac	5	2	7
Myocardial infarction	(4)	(1)	
Heart failure	(1)		
Arrhythmia		(1)	
Upper GI bleeding	2		2
Perforated ulcer	1	1	2
Colon gangrene		1	1
Stroke	1		1
Paraplegia	1		1
Dye allergy		1	1
Multiple-system failure	1		1
TOTAL	11	5	16

TABLE 7
Complications

	No.
Cardiac	27
Myocardial infarction	11
Congestive heart failure	10
Arrhythmia	6
Renal failure	5
Bowel obstruction (operated)	5
Embolus (clot or atheroma)	4
Hemorrhage (operated on)	4
Stroke	3
Deep venous thrombosis	3
Pulmonary embolus	1
Paraplegia	2
Upper GI bleed	2
Perforated ulcer	2
Colon gangrene	1
Pancreatitis	1
Spleen injury (splenectomy)	1

Discussion

All abdominal aortic aneurysms can rupture; the risk of rupture is always related to the size of the aneurysm (8). Small aneurysms less than 4 cm in diameter have a low risk of rupture, especially if the diameter of the aneurysm is less than twice the diameter of the patient's normal aorta (9). However, a small aneurysm is often an early stage of a large one and must be followed up. Small aneurysms grow an average of 0.4 cm a year but can enlarge more rapidly (10).

Aneurysms larger than 4 cm entail a greater risk of rupture, the risk rising sharply as size increases. In an autopsy study of unoperated-on abdominal aortic aneurysms, Darling et al (8) found that 23.4% of aneurysms 4.1–5.0 cm had ruptured and that 25.3% of aneurysms 5.1–7.0 cm had ruptured. An 8-cm aneurysm has a greater than 75% risk of rupture within 5 years (11). Although enlargement during observation implies imminent rupture, any aneurysm can rupture at any time, and it is a fallacy to think an aneurysm will enlarge prior to rupture. Since the only effective treatment of an aneurysm is resection, a physician advising a patient with an abdominal aortic aneurysm must balance the patient's medical history and the size of the aneurysm with the risk of operation.

In our study, during the decades from 1963 through 1984 the operative mortality for resection of an abdominal aortic aneurysm declined from 8% to 2% when death from all causes is included. If the one death of dye allergy is excluded, the operative mortality in 1975–1984 was 1.4%. The improvement in outcome is attributed to several factors: physician's awareness of and improved diagnostic techniques for heart disease, availability and low risk of coronary bypass operation for coronary artery disease, monitoring cardiac function during and after operation with the Swan-Ganz catheter, preoperative storage of autologous blood and the ability to intraoperatively retransfuse shed blood, improved graft and suture material, and, most important, a group of experienced surgeons working as a team.

In 1957 Shapiro (12) found that in an autopsy study an equal number of patients died with an intact aneurysm of unrelated causes as died of a ruptured aneurysm and advised caution in recommending surgery for aneurysms less than 6 cm in diameter or for older patients with cerebral and coronary artery disease. In the ensuing years it was generally agreed that 6–7 cm was the size that required surgery. At the time of

Shapiro's report the risk for resection of an aneurysm was 7% or greater. Today, an operative risk of 2% or less permits expanding the indications for surgery, and we now advise operation for 5-cm aneurysms and frequently for smaller ones. Our series includes patients with heart disease, hypertension, diabetes mellitus, pulmonary disease, and renal insufficiency, and these risk factors should not preclude aortic resection.

In the past, older patients with an aneurysm have been denied surgery based solely on their age. We previously reported (13) that octogenarians can undergo resection of an aneurysm with an acceptable risk, especially when compared to the outcome once rupture occurs. Furthermore, after surgery older patients returned to a useful existence. Today, age is no barrier to recommending surgery for patients threatened by an abdominal aortic aneurysm.

All patients with nonresected aneurysms must be followed up. Ultrasound is frequently used to follow the size of the aneurysm because of ease of testing, safety, and cost considerations. However, we have found computerized tomography to be more accurate. Magnetic resonance imaging is new to our hospital, but preliminary observations suggest it may become the test of choice.

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Ventricular Tachyarrhythmias during Placement of Pulmonary Artery Catheters in Two Patients with Recurrent Ventricular Tachycardia

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Abstract

Surgery is being performed with increasing frequency in treatment of patients with intractable ventricular arrhythmias (1–3). Because these patients generally have low cardiac output and global left ventricular dysfunction, the ability to monitor left-sided filling pressures and measure serial cardiac outputs is valuable during the induction, postbypass, and intensive-care recovery periods (4). To this end, we place flow-directed pulmonary artery catheters prior to induction of anesthesia in patients undergoing electrophysiologically guided endocardial resections. Recent studies have reported an incidence of up to 50% of ventricular arrhythmias (three or more consecutive PVCs) during insertion of pulmonary arterial catheters in critically ill patients (5–7). These arrhythmias generally respond to repositioning of the catheter or administration of lidocaine. Patients with intractable ventricular tachycardia may not respond to these maneuvers (8,9). We report on two patients with PA-catheter-induced, sustained ventricular tachycardia which caused hemodynamic compromise.

Case Reports

Patient 1. Five years after transmural myocardial infarction, a 57-year-old white man had several episodes of ventricular tachycardia despite antiarrhythmic drug therapy. Cardiac catheterization revealed total occlusion of the left anterior descending artery, 80% occlusion of the posterior descending artery, and a large apicoinferior aneurysm. Electrophysiologic mapping revealed an arrhythmogenic focus in the inferior aspect of the midinterventricular septum. Various antiarrhythmic regimens were ineffective; pacemaker therapy was ruled out because of the excessive rates required to control the arrhythmia. Prior to scheduled surgical treatment for the tachyarrhythmia, a No. 6 French quadripolar-transvenous pacemaker was inserted via a peripheral arm vein, in the electrophysiology catheterization lab.

The patient arrived in the operating room medicated with morphine and scopolamine. Monitoring with a five-lead electrocardiogram and percutaneous radial arterial and peripheral venous lines was established. Right internal jugular approach to the central circulation by Seldinger technique was used for passage of a No. 7 French pulmonary arterial (PA) catheter. As the catheter entered the right ventricle, the rhythm immediately changed to rapid ventricular tachycardia of 130 beats per minute, and the patient became hypotensive. The catheter was immediately withdrawn to 20 cm. After 3 min of hypotension, with mean blood pressures of 30 torr, the rhythm converted to sinus with rapid overdrive ventricular pacing via the indwelling pacing

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wire. The catheter was passed to the right atrium and later advanced into the pulmonary artery after the chest had been opened and the heart exposed. The operative and recovery periods were otherwise uneventful.

Patient 2. Fourteen years after myocardial infarction, a 61-year-old white woman developed symptomatic congestive heart failure and ventricular tachyarrhythmias. After surviving one episode of ventricular fibrillation, the patient underwent electrophysiologic studies. Pharmacologic interventions were ineffectual in controlling the ventricular tachycardia, making her a candidate for surgical treatment. Cardiac catheterization revealed total occlusion of the left anterior descending artery, an ejection fraction of 10% and an apical aneurysm. In the catheterization lab a No. 6 French quadripolar catheter was placed in the patient via a peripheral vein.

The patient arrived in the operating room medicated with morphine and scopolamine. Prior to induction of anesthesia, five-lead electrocardiogram and peripheral venous and radial arterial access were established. Seldinger technique was employed to gain central venous access via the right internal jugular vein, and the PA catheter was inserted. As the catheter tip entered the right ventricle, ventricular tachycardia developed, and the patient's blood pressure fell to a mean of 40 torr. The catheter was withdrawn to 20 cm, and cardioversion terminated the dysrhythmia. The patient was monitored with central venous pressure until placement of a left atrial catheter upon termination of bypass. The remainder of the operative and recovery periods was uneventful.

Discussion

The placement of PA catheters may produce ventricular arrhythmias which are generally transient and not of hemodynamic significance (10). Patients arriving for surgical therapy of ventricular tachyarrhythmias have already been proven refractory to long-term pharmacologic therapy and may prove difficult to treat in the operating room. If antiarrhythmic therapy is used to terminate ventricular tachycardia, the rate of the arrhythmia may increase (11). Information concerning the patient's antiarrhythmic pharmacologic history will be available in the electrophysiologic data obtained preoperatively. Intraoperative electrophysiologic studies may be interfered with by antiarrhythmic medications, thus providing a relative contraindication to their use (12).

We reviewed our experience with 165 consecutive, operative, electrophysiologically guided endocardial resections performed at this institution between 1978 and 1984. Of these, 144 patients had PA catheters and 21 had central venous catheters placed prior to induction of anesthesia. The two cases reported herein were the only two cases of sustained ventricular tachyarrhythmias associated with catheter placement. We believe both of these cases to be associated with "normal" passage of the catheter as judged by the operator and not due to the catheter hanging up in the apex of the right ventricle.

Precautions. We therefore recommend that certain precautions be taken prior to placement of PA catheters in these patients.

First, all patients should be monitored continuously with electrocardiogram and intraarterial pressure monitoring during PA catheter placement.

Second, personnel and equipment should be sufficient for immediate performance of rapid pacing or cardioversion if arrhythmias develop. Equipment should include all supplies necessary to deliver positive-pressure ventilation with high inspired oxygen tensions, defibrillator with which members of the anesthesia team are familiar and have checked for proper functioning, and pharmacologic support for the circulation in the event of hemodynamic compromise. Familiarity with and availability of pacer modalities such as PA catheters with pacemaker ability, transvenous pacers (patients who need surgical correction of ventricular arrhythmia often arrive in the operating room with transvenous pacemakers in place), and the control boxes for these pacemakers should also be required prior to insertion of PA catheters.

Third, an alternate approach is placement of the PA catheter within a sterile, plastic sheath and passage of the catheter into the central venous circulation prior to induction. After the heart has been surgically exposed, the anesthesiologist can advance the catheter into the pulmonary artery without disturbing the surgical field. If a life-threatening arrhythmia develops in this setting, therapy is simplified. The major drawback of this technique is the inability to measure cardiac output or monitor left-sided filling pressures during induction of anesthesia.

Fourth, fluoroscopy could be used, either in the operating room or in the catheterization laboratory at the same time that the electrode catheter is inserted, to properly guide the catheter through the right ventricle and thus avoid arrhythmias caused by "hanging up" of the catheter at the apex.

In conclusion: although the risk of sustained PA-catheter-induced ventricular tachycardia is small, such an event may have grave consequences and require special precautions.

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Relation of Atrial Flutter to Graves' Disease

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The cardiovascular complications of hyperthyroidism have intrigued physicians for decades. Sinus tachycardia, paroxysmal atrial fibrillation, and increased voltage of the QRS complexes are frequently found on the electrocardiogram of the hyperthyroid patient (1). PR interval prolongation, reversible bundle branch block, ventricular tachycardia, ventricular fibrillation, atrial flutter with 1:1 conduction, and complete heart block with Morgagni-Adams-Stokes syncope have been reported (2–5). Disturbances of atrioventricular conduction are less common but potentially more serious (2). Atrial flutter is not commonly associated with hyperthyroidism. Standard textbooks mention that atrial flutter occurs in thyrotoxicosis, but none of the major textbooks provide a definite reference.

We report here a recent clearly documented case of this arrhythmia. Furthermore, we have echocardiographic data which shows that the left atrium was not enlarged and left ventricular hypertrophy was not present. The older literature obviously does not have these data; whether patients who exhibited this arrhythmia many years ago did, in fact, have left atrial enlargement or even rheumatic heart disease will never be answered. We report a case of atrial flutter induced by thyrotoxicosis.

Case Report. A 38-year-old man had been diagnosed to have Graves' hyperthyroidism at age 32. Serum T_4 was 20.2 mg% (normal 4.5–12.5) and T_3 was 925 ng% (normal 80–180). Electrocardiogram revealed sinus tachycardia at 150/min. His initial treatment consisted of methimazole, 20 mg three times a day, and propranolol for 6 months, followed by a second 5-month course. A year after discontinuation of therapy the patient was in sinus rhythm with a T_4 of 12.8 mg% and T_3 of 261 ng%. On May 5, 1983, at age 38, he was admitted to the surgical service for rhinoplasty.

On physical examination he was tremulous and diaphoretic and had blood pressure of 130/80 and a pulse of 150/min. The thyroid gland was firm, nontender, and small. His chest was clear and no abnormalities were found in cardiovascular examination or chest x-ray. Serum calcium was normal and he was not being treated with digoxin. Electrocardiogram revealed atrial flutter with 2:1 atrioventricular block and tall voltage of the QRS complexes. Thyroidal iodine 131 uptake was 44% at 24 hr. He again received methimazole, 20 mg four times a day, propranolol, and Lugol's solution, and ventricular rate decreased to 75–100/min with no change in rhythm. Echocardiogram showed normal left atrial size, and left ventricular hypertrophy was not present. The patient was cardioverted (75 joules) and treated with quinidine sulfate and digoxin. After treatment with 6.4 mCi iodine 131, methimazole and digoxin were discontinued. He remains euthyroid and in sinus rhythm.

Discussion. The ECG manifestations of hyperthyroidism have received considerable attention. A wide variety of nonspecific ECG abnormalities have been reported, but disagreement remains about the relation of such abnormalities to the thyrotoxic state (1).

Investigators have attributed the etiology of atrioventricular conduction disturbances to digitalis, infection, hypercalcemia, or a thyrotoxic

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myocarditis. Current data support the concept that thyroxine has a direct effect on the atrial tissue independent of the autonomic nervous system, causing an increase in sinus node automaticity and a decrease in the effective refractory period of the atrium (2). Thyroid hormone markedly facilitates impulse transmission through the atrioventricular conduction system (3).

The predominance of atrial arrhythmias in hyperthyroidism is understandable in view of the enhanced conduction velocity and shortened refractory period. Atrial flutter associated with hyperthyroidism (6) is an uncommon arrhythmia with a low incidence. All patients with hyperthyroidism, especially the elderly, should have electrocardiograms performed at the time of diagnosis and periodically during therapy.

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Nerve Blocks: An Anesthesiologist's Approach to the Relief of Cancer Pain

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The anesthesiologist with some experience in pain control can perform a number of nerve blocks to relieve the pain of cancer, using local anesthetics, neurolytic agents, and steroids. Too many patients needlessly undergo unnecessary pain while receiving inadequate medication.

Interruption of the pain pathways may be temporary or permanent, chemical or physical. Chemical interruption consists of nerve blocks, where either a local anesthetic agent or antiinflammatory drug or neurolytic compound is used. Nerve blocks for cancer usually depend on peripheral nerve blocks, sympathetic blocks, antiinflammatory blocks, or subarachnoid neurolytic blocks. Large plexuses are blocked only when absolutely necessary because the sensory and motor deficit achieved—a numb, heavy, paralyzed arm, with no proprioception—is too great a price to pay for pain relief. Usually, somatic nerves are blocked more distally.

Cervical plexus blocks are performed for metastatic lesions in the neck causing pain in the neck and occipital pain. Bupivicaine ½% is used, and can be frequently repeated. If the patient feels the relief is short-lived, equal parts of bupivicaine and absolute alcohol can be used for longer periods of relief. Occasionally, 40 mg Depomedrol may be added to the local anesthetic for more extensive relief, but pain may recur after the local anesthetic wears off and before the steroid takes effect. In other cases of head and neck pain from metastatic carcinoma, the trigeminal nerve can be blocked at the maxillary and mandibular divisions, with bupivicaine or alcohol. The maxillary nerve may be reached via the greater palatine foramen and pterygopalatine fossa; the madibular nerve can be blocked via the conventional dental route against the ramus of the mandible (1).

Intercostal pain following thoracotomy, which becomes intractable, responds well to intercostal nerve blocks, first using ½% bupivicaine, repeating it daily, (and adding depomedrol, if desired) and later, if necessary, making the result more permanent, with absolute alcohol. Some have used the cryocautery needle for this purpose, achieving about a month of relief, until nerve healing occurs. In intercostal blocks, always block the space above and below, because of interdigitation of nerves. Remember, too, that the highest blood levels of local anesthetic, apart from intravenous injections, occur with intercostal blocks.

Ilioinguinal and iliohypogastric nerves are injected for severe groin pain, occasionally with success. Before peripheral neurolysis, a test block must be done with local anesthetic to ensure that the correct nerve is blocked, that relief does occur, and that side effects of analgesia, loss of proprioception, and so on, are acceptable to the patient on a more permanent basis.

The entire sympathetic nervous system is easily accessible in three areas, the stellate ganglion, the celiac plexus, and the lumbar sympathetic chain, which can all be reached with a needle.

Patients with cancers of the head and neck, upper extremitities, and upper chest that cause pain resembling causalgia or sympathetic dystrophy—pain that is burning, vague, not easily localized, and referred—frequently respond to stellate ganglion block, performed with local anesthesia. The response lasts far longer using local anesthetic than the duration of action of the local

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anesthetic; relief can be obtained for days and weeks, rather than for hours. Under special circumstances, and if the patient accepts acquiring a permanent Horner's syndrome in exchange for pain relief, the ganglion can be blocked with absolute alcohol or phenol 6%. Bilateral stellate ganglion blocks should never be done, as the recurrent laryngeal nerve, which is very close, may be bilaterally blocked, causing loss of airway. One-sided stellate block with one-sided recurrent laryngeal block would only cause temporary hoarseness (2).

Pain in the pelvis and lower extremities frequently responds temporarily to lumbar sympathetic blocks, performed with anterior-posterior and lateral x-ray control (2). Sympathetic effect will be evidenced by warming of the extremity, relief of pain, cessation of sweating of the limb, and Horner's syndrome (if the procedure is stellate ganglion block). Complications of stellate block are pneumothorax and intravascular injections.

Postherpetic neuralgia, which may follow radiotherapy, may respond to sympathetic block if diagnosed in the first 12 weeks. Later, the neuraxis undergoes changes, and treatment is more difficult. At this stage, various combinations of antidepressants, tranquilizers, and antiepileptic drugs are offered.

Celiac plexus blocks (3, 4) are great pain relievers for patients with carcinoma of the pancreas and retroperitoneal metastases. We have used them for postlaparatomy extensions of carcinoma of stomach, gall bladder, and retroperitoneal neurofibromatosis, with sarcomatous change (Table 1).

TABLE 1
Celiac Plexus Block

	No. of cases	Good results
Carcinoma of pancreas	89	76
Chronic pancreatitis	51	36
Crohn's disease	5	2
Retroperitoneal metastases	2	1
Carcinoma of stomach & gall bladder	2	2
Abdominal pain following multiple laparotomies	1	0
Total	150	117

The patient must be well hydrated and in electrolytic balance before the procedure. Absolute alcohol is the agent of choice. The block is performed with x-ray control, anteroposterior and lateral (Fig.). The injection is painful; the patient is given morphine and Valium for sedation. Only one side at a time is blocked because of the severity of the postural hypotension. The second side is blocked a few days later. Pain relief is marked, and usually lasts about 9 months; the blocks can be repeated when pain recurs. Most patients ask why this treatment was not offered sooner. One or two celiac blocks make the patient alert and drug-free (if he or she is not yet addicted). The prognosis for the cancer remains the same, but apart from being free of pain, the patient goes home alert, without constipation, and free of narcotics.

Complications of celiac plexus block include postural hypotension (the patient should walk with elastic stockings for the first 48 hours) and penetration of kidneys by the needle (5). We have seen two cases of pleural effusion. Pneumothorax has been reported. Numbness or hyperesthesia of the groin may follow spillage of alcohol on somatic nerve L1.

Transsacral neurolytic blocks have been performed for severe perineal pain for metastatic spread of rectal carcinoma. Perineal sensory innervation is mainly from S4. This nerve is accessible via the posterior sacral foramen of S4, located 1 cm lateral and 1 cm cephalad to the sacral cornu on each side; it can be palpated in thin subjects. The needle is advanced 1 cm into the foramen, and 2.5 ml of 6% phenol is injected into both sides. This can be repeated whenever the pain returns, usually in a few weeks.

Epidural Steroids. We have had a surprising amount of success for malignant radiculopathy causing backache, pain in pelvis, and pain along the femoral nerve and sciatic distribution with epidural steroids. We achieved good pain relief in 18 of 22 patients (Table 2). Apparently, the steroids produce an antiinflammatory and antiedematous action, thus freeing the nerve roots, at least partially, from the pressure exerted by the metastases. We have seen relief for up to 3–4 months, and further relief following another course of treatment. We have given 80 mg sterile methylprednisolone acetate suspension (Depo-Medrol) epidurally every 3–4 days for 3–4 injections. If we see no improvement, we stop the course of injections. At first the pain increases because of the irritant nature of the material, but after about 8 hours, the pain subsides.

Subarachnoid Neurolytic Blocks. When epidural steroids no longer work and when the pain is intractable, the patient is going downhill and bedridden, subarachnoid neurolytic block should be

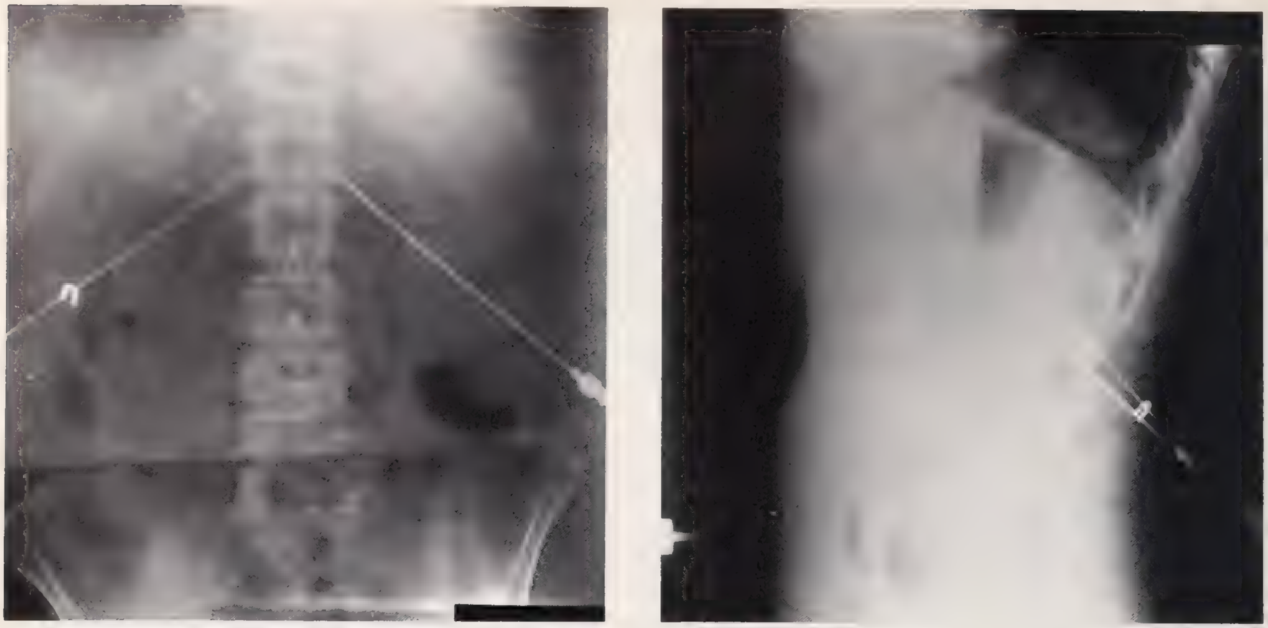

Fig. Anteroposterior and lateral views of celiac plexus block, bilateral. Tip of needle is at L1, anterolaterally. Marker is on needle on right side.

considered. The patient and the family should understand that apart from pain relief, there may be side effects, such as numbness, motor weakness, and loss of bladder and/or rectal continence (occasionally the patient is already incontinent or has a colostomy or cutaneous uretrostomy). If the patient is still willing to risk some of these complications in exchange for pain relief, he or she should sign to this effect before undergoing the procedure.

We have performed neurolytic blocks on a number of patients (Table 3). One patient with an advanced osteogenic sarcoma of the pelvis (for which he later had a hemicorporectomy) had excellent pain relief. Another patient, with retroperitoneal neurofibrosarcoma, had no relief at all. A patient with pelvic pain following uterine carcinoma became pain-free, but was unhappy about a feeling of floating on air while in bed because she had lost sensation and proprioception of the pelvic region.

Phenol 6% in glycerine is preferable to absolute alcohol in the subarachnoid space. Somehow, the alcohol becomes diluted by 150 ml of cerebrospinal fluid and in a few days, the effect wears off. Phenol in glycerine is viscous and seems to have a much longer-lasting effect. Remember that phenol in glycerine is hyperbaric and alcohol is hypobaric. The patient must be positioned so that the dorsal sensory roots of the particular dermatomes involved will be bathed by the solution. If alcohol is being used, the dorsal root and dermatome to be injected must be uppermost. If phenol in glycerine is injected, the required dermatome must be most dependent. 1/10 ml is injected at a time. The patient's cooperation is required to describe the site of the tingling sensations, so that the table or the patient's position

TABLE 2

Epidural Steroid Injections

Carcinoma	No. of Pts.	No. of injections	Good pain relief
Gynecologic	8	28	6
Rectal	7	24	6
Breast	3	12	3
Prostatic	2	10	2
Other	2	6	1
Total	22	80	18

TABLE 3

Subarachnoid Neurolytic Block

Carcinoma	No. of pts.	No. of injections	Good pain relief
Gynecological	4	8	3
Rectal	4	6	2
Prostatic	3	5	2
Bone (primary sarcoma)	2	3	2
Bone (metastatic)	5	8	3
Retroperitoneal	2	3	0

can be adjusted until 1 ml has been injected. If insufficient relief has been obtained, further injections can be given at a later date. Care must be taken to keep the solution away from the opposite side and away from the motor roots. This can best be done by meticulous positioning and by placing the needle very superficially through the dura. In fact, if the flow of cerebrospinal fluid is brisk, the needle should be gradually withdrawn until the flow is slow, and the bevel sits just under the dura.

Unfortunately, a large number of patients will not respond to the treatments mentioned. For them, the most effective treatment is destruction of the pain pathways (7). The closer the ablation to the receptor organ, the more effective the procedure, because of the branching of the pain pathways centrally. Pain fibers enter the spinal cord at the posterior nerve root, ascend for a variable number of segments, then cross to the opposite side to take up their position, anterolaterally in the spinal cord—the spinothalamic tract. Section of the spinothalamic tract gives altered sensation from a few segments below the section to the end of the spinal cord at S5, the anus. Heat and cold sensations, too, will be destroyed. Occasionally, touch from the opposite end will be effected. Crossover in the spinal cord occurs only to 90% of pain fibers; 10% of pain fibers never cross. In some people, the percentages vary and occasionally, after spinothalamic section, ipsilateral analgesia has occurred because there has been minimal crossover. This is the reason for some of the failures. When effective, results are longlasting.

Instead of choosing a neurosurgical procedure, which entails increased risks in the surgery and the anesthesia for patients who are probably already very sick, percutaneous cordotomy can be performed. Bilateral surgical cordotomy is not recommended because of the need to rotate the cord at surgery in order to do the other side. This may cause muscle weakness and bladder and respiratory problems. Respiratory problems that follow bilateral cordotomy are known as Ondine's syndrome, which is sleep apnea. Poor respiratory reserve increases the mortality rate in those requiring cordotomy. The FEV should be 1.5 L, although the procedure is sometimes performed when the FEV is as low as 1L. A second cordotomy is less successful because of fibrosis following the first. Sometimes, weakness for a few days due to cord edema is relieved by steroids.

Pituitary injection of alcohol by stereotaxis is occasionally used for treating hormone-dependent tumors. Complications are rhinorrhea, hypothalmic coma for a few days, diabetes insipidus, hypopituitarism, steroid deficiency, hypothermia, and hyperphagia.

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Arterial Injuries of the Lower Limb with Associated Fractures

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Abstract

From January 1979 to December 1983, 45 patients were treated for fractures and associated peripheral vascular injuries of the lower extremity. Vascular reconstruction, after appropriate arteriography, was attempted in all but 3 of these patients. The most common method of repair was autogenous vein interposition graft (23 patients) followed by direct end-to-end anastomosis (10 patients). Thirty-two limbs (75%) were saved, and 12 patients (25%) underwent eventual amputation. Long periods of ischemia (over 10 hr), extensive local soft-tissue and bony damage, severe concomitant head, chest, or abdominal injury, and failure or inability to repair simultaneous venous injury are the major factors that contribute to therapeutic failures.

Despite the large number of previous reports (1–6), the management of patients with fractures and associated peripheral vascular injuries remains controversial.

This report describes our experience treating 45 patients who sustained this complex type of injury. The patient population is analyzed with respect to the mechanism of injury, the extent of soft-tissue damage, the location of the associated fractures, and the types of vascular reconstruction performed.

Patients and Methods

During the 5-year period from January 1979 to December 1983, 45 patients with arterial injury of the lower extremity and associated fractures (Tables 1, 2) were treated in our department. Patients ranged in age from 11 to 68 years. Blunt trauma was responsible for arterial injury in 27 patients (60%) and gunshot wounds in the remaining 18 patients (40%) (Table 2). Fractures of the femur were the most common (32/45). Eight patients had tibia-fibula fractures, and 2 suffered pelvic fractures.

Appropriate arteriography was performed in all patients in the x-ray department or intraoperatively. At surgery, complete arterial disruption was found in 35%, and 65% had intimal and medial tears with posttraumatic thrombosis. The most common technique of arterial repair was either interposition of autogenous vein graft (51%) or direct end-to-end anastomosis (22%). Simple evacuation of a hematoma with fasciotomy was performed in 2 patients with no primary revascularization. Three patients underwent primary amputation. Thrombectomy was performed on one patient. Fasciotomy as an adjunct to revascularization was performed in 15 patients. There were 15 major venous injuries associated with the 45 arterial injuries (Table 3).

Results

There were 2 deaths in our series, an incidence of 4%. Twelve amputations were performed (26%). According to the level of vascular disruption, 4 of the amputations were for femoral artery

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TABLE 1
Amputations by Site of Arterial Injury in 45 Patients

Site	Patients No.	Patients (%)	Amputations No.	Amputations (%)
Iliac	2	(4)	—	
Femoral	21	(47)	4	(19)
Popliteal	18	(40)	5	(28)
Tibioperoneal	4	(9)	3	(75)

TABLE 3
Results of Repair vs. Ligation in Treatment of Associated Major Venous Injuries in 45 Patients

	No. of patients	Good results No.	Good results (%)
Vein repaired	6	5	(83)
Vein ligated	9	5	(56)
Total	15	10	(67)

injury, 5 for popliteal artery injury, and 3 for extensive lesions of the tibioperoneal trunk. The ligation of a major vein resulted in the amputation of the limb in 4 of 9 patients, but when the vein was repaired only 1 limb of 6 was lost (table 3).

Thirty-three limbs (71%) were salvaged. Results in 9 patients were considered only fair, because residual nerve and/or orthopedic problems prohibited full functional recovery, but results in the remaining 24 patients were functionally excellent.

Discussion

In cases of complex trauma to the lower extremity, the association of fractures and vascular injuries requires prompt, multidisciplinary surgical management (7). The rapidity of restoration of venous and arterial flow is an important factor in limb salvage. In our series the amputation rates rose from 9% to 30% when this time interval exceeded 10 hr (2, 3, 8).

When time permits, arteriography can be performed in the radiology department. If there is urgency regarding the state of circulation, preoperative arteriography can be performed while the patient is on the operating table. Intraoperative arteriography may help delineate more distal lesions not visualized by preoperative femoral arteriograms, especially in cases of multilevel trauma (Fig. 1) (7).

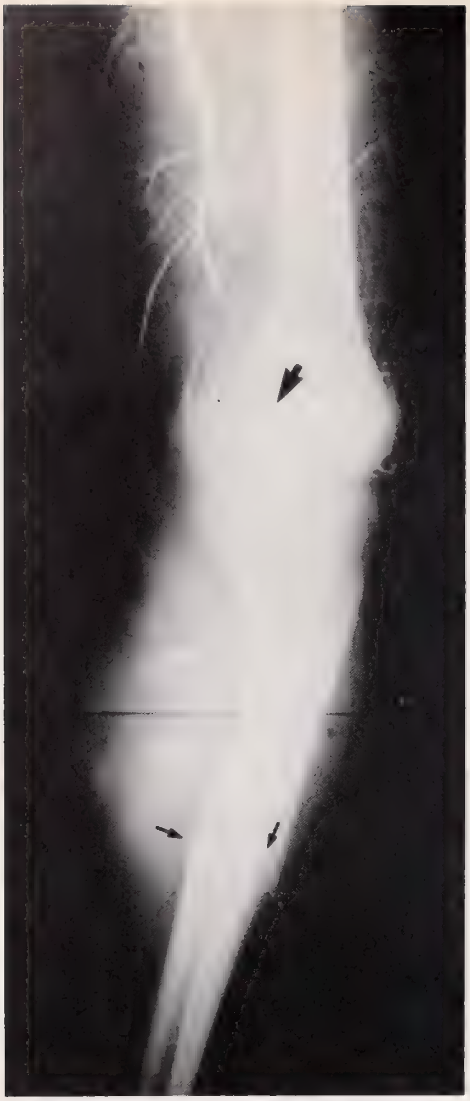
In complex limb trauma, optimal timing is the basic tenet for successful operation. Judging from our and others' experience (5, 7, 9), prompt stabilization of the fracture site should be the first therapeutic measure. We believe that external stabilization is preferrable; its benefits include ease and rapidity of application at a site distant from vascular lesions and easy postoperative assessment of circulatory and wound status (5, 8, 10). In the case of long-term, preoperative, limb-threatening ischemia, immediate restoration of arterial flow can be obtained by insertion of a temporary Silastic shunt prior to bony stabilization. This procedure was followed in one patient in our series and proved to be a valuable adjunct.

After adequate stabilization, the simplest and most direct form of arterial repair, debridement and direct reanastomosis (Fig. 2), is the treatment of choice (7). In complex limb trauma the arterial injury may often be more extensive than appreciated (9). In 64% of our cases the lesion was too extensive to be reconstructed by direct anastomosis, and bypass grafting was necessary (9). In such cases, autogenous saphenous vein seems to be the material of choice—although there is some disagreement in the literature (11)—because of its better resistence to infections and the ease of harvesting the short segments usually needed. Saphenous vein was used in 80% of the bypasses we performed. Prosthetic grafts were used in 6 cases, but infection ensued in 4 of these, requiring graft removal and subsequent amputations.

When there is injury to a major vein, venous repair should always be performed primarily, if at all possible (6, 7, 12). Unfortunately, the repair of severe venous injury may be impossible, leaving ligation or synthetic grafting (11) as the

TABLE 2
Results in 45 Patients by Mechanism of Injury

	Patients No.	Patients (%)	Results Good	Results Fair	Results Amputation	Results Death
Gunshot	17	(38)	10	4	3	—
Shotgun	1	(2)	—	—	1	—
Blunt trauma	27	(60)	14	5	8	2



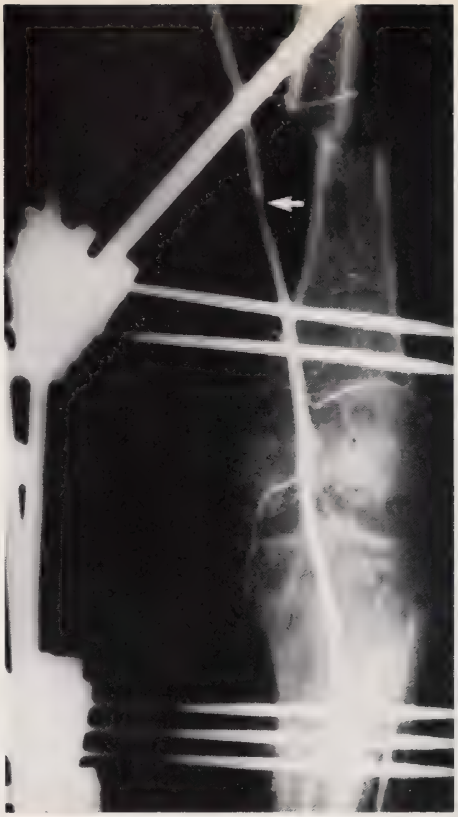


Fig. 1. Left Multiple gunshot wounds of left leg with superficial femoral artery thrombosis. Note bullet at knee level (single arrow) and multiple fragments of another bullet in leg with associated fracture (arrows).

Fig. 2. Right Postoperative arteriogram in patient with multiple bone fractures of left leg. Arrow shows level of end-to-end anastomosis.

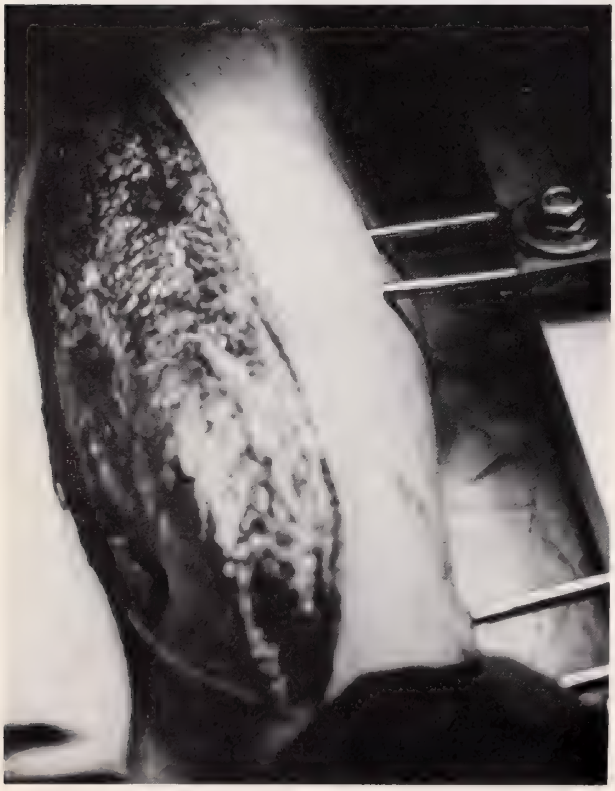
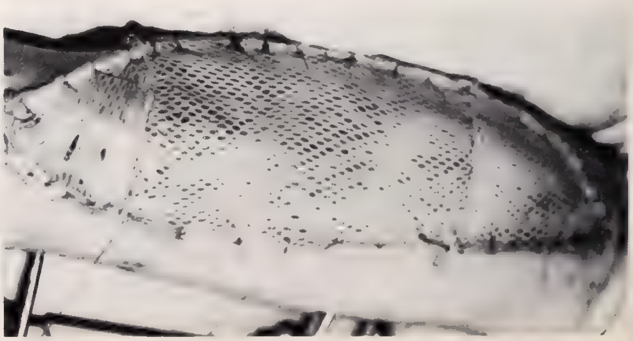


Fig. 3. Left Fasciotomy performed for acute onset of posterior compartment syndrome after external fixation and vascular reconstruction. Note marked muscle edema. Muscles are still viable.

Fig. 4. Right Same patient. Skin graft performed 3 days later.



only alternative. Although the population size of our series (Table 3) is small for generalization, repair seems clearly preferable to ligation (3).

Four-compartment fasciotomy of the leg (1, 13, 14) is indicated when vascular repair has been considerably delayed, venous compromise is severe, or there is any doubt about increasing compartment tissue pressures (Fig. 3) (2). If fasciotomy is not performed at the initial operation and considerable edema develops, fasciotomy must then be performed immediately. In these cases coverage of the exposed muscles with early skin graft 3–7 days later results in a significant shortening of hospitalization (Fig. 4) (4).

In our series, as in others, the presence of extensive remote injury plays an important role (3). The two deaths that occurred were in patients who suffered severe blunt trauma with injury of the lower popliteal and the tibioperoneal artery and who also had extensive multisystem trauma.

The overall amputation rate was 26%. Although there was no significant difference in the amputation rate by mechanism of injury (Table 2), the level of vascular injury was significant. In general, the more distal the lesion, the worse the prognosis (Table 1). Three of these twelve amputations were performed primarily in patients in whom the gravity of the lesion or whose general condition (age, associated injury) contraindicated any attempt at repair.

The association of fractures with arterial injuries carries a worse prognosis than arterial injuries alone (3), mainly because associated distant injuries are frequent. Impaired function following treatment of vascular trauma of the extremities depends in large part on associated injuries. Despite successful revascularization, poor limb function can still result from concomitant nerve, soft-tissue, or joint injury (3, 8, 9). This can be minimized, when possible, by appropriate adjunctive procedures and aggressive physical therapy.

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Plasma Insulin Response to Oral Glucose in Alcoholics with and without Exocrine Pancreatic Hypersecretion

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Abstract

Exocrine pancreatic function and plasma insulin response to oral glucose were studied in 16 alcoholics who had no clinical or laboratory evidence of hepatic disease. The tests used were the secretin-cholecystokinin (S-CCK) test and the oral glucose tolerance test (OGTT); the latter was also performed in 8 control subjects. The S-CCK test results were not normal in 12 patients, 7 of whom were bicarbonate and protein hypersecretors. The insulin response was decreased in 10 patients (5 with, and 5 without, exocrine hypersecretion). All but one of the 16 alcoholics showed exocrine and/or endocrine pancreatic dysfunction. Thus, these tests are complementary in studying pancreatic function in alcoholics, and together contribute to a more precise diagnosis. Low levels of plasma insulin in the absence of detectable alterations in OGTT in alcoholics could be a precocious indicator of pancreatic dysfunction.

Alcoholism, a serious public health problem in almost all countries, in Brazil represents 95% of all drug abuse (1). The most popular alcoholic beverage is cachaça (a cheap beverage distilled from sugar cane, with a high ethanol content, approximately 300 gm/L).

Long-term and immoderate alcohol intake can damage social, mental, and organic health. Moreover, alcoholism is accepted as the main etiologic agent of chronic pancreatitis (2, 3).

Three patterns of exocrine pancreatic bicarbonate secretion have been described in alcoholics (4): normal, hypersecretion, and hyposecretion. The latter has been extensively studied and is frequently associated with pancreatic fibrosis (5). Exocrine pancreatic hypersecretion represents the secretory pattern of minimal pancreatic inflammatory pathologies as a consequence of duct reduplication, hypertrophy, and hyperplasia (6, 7).

Performing the secretin tests in chronic alcoholics without clinical or laboratory evidence of hepatic or pancreatic disease, Neves et al (8, 9) found, in 30% of the subjects studied, exocrine bicarbonate hypersecretion after stimulus with secretin, and exocrine hypersecretion of both bicarbonate and protein after secretin-cholecystokinin (S-CCK). It seems probable that this hypersecretory state would be the first detectable evidence of early pancreatic dysfunction resulting from chronic and immoderate ethanol intake. Thus hyper- and hyposecretory patterns are clear indicators of pancreatic dysfunction. On the other hand, a normal exocrine pattern in chronic alcoholism could signify either a real normal physiologic state or a possible intermediary phase between hypersecretion and final, irreversible hyposecretion. The study of endocrine secretory behavior in alcoholics with this normal exocrine pattern would be of help in improving the accuracy of diagnosis of pancreatic dysfunction.

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Decreased glucose tolerance and impaired insulin secretion have in fact been demonstrated in chronic pancreatitis (10, 11), but conflicting data have been reported on the effects of long-term ethanol intake on glucose tolerance and insulin secretion in alcoholics without established chronic pancreatitis. Both increased (12) and decreased (13) insulin secretion have been described following long-term ethanol administration or intake.

In this study, the plasma insulin response after oral glucose in chronic alcoholics, with and without pancreatic hypersecretion, is examined in an attempt to clarify both exocrine and endocrine behavior in these patients.

Methods. We performed the oral glucose tolerance test (OGTT) in 24 adults of both sexes divided into two groups. The control group was made up of 8 healthy volunteers (3 males and 5 females), aged 22 to 46 (mean, 32 years), weights of 42 to 67 kg (mean, 53 kg), and without any history of endocrine or hepatopancreatic disease. The group of alcoholics comprised 16 subjects (15 males and 1 female), aged 28 to 55 (mean, 35 years), weights of 46 to 80 kg (mean, 61 kg), without any clinical evidence of pancreatic or hepatic disease, and with a daily average ethanol intake of 200 gm for an average of 13 years, the minimum being 100 gm/day for 4 years.

Laboratory tests (protein electrophoresis, prothrombin time, bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and amylase) were normal in all subjects.

Both groups were put on a diet of 100 gm of carbohydrate for 3 days before determining OGTT. On the fourth day, 100 gm of oral glucose was given and blood was collected at 0, 30, 60, 120, and 180 minutes. Plasma glucose and insulin levels were determined by a glucose oxidase method (14) and by radioimmunoassay (15, 16). Total integrated responses (TIR) to plasma glucose and insulin were calculated from the sum of the products of the mean serum concentrations during each time period, multiplied by the number of minutes of the period (17).

TABLE 1

Results of Secretin-CCK Duodenal Aspirate in Alcoholic Study Groups (Mean ± SE)

	Hypersecretor (N = 7)	Nonhypersecretor (N = 9)	p
HCO_3^-			
Total output (mmol/45 min)	17.7 ± 1.0	5.5 ± 0.9	<0.01
Maximum concentration (mmol/l)	85 ± 5	54 ± 3	<0.05
Volume			
Total (ml/kg)	3.5 ± 0.3	1.8 ± 0.2	<0.01
Protein			
Total output (mg/45 min)	294 ± 55	150 ± 32	<0.05

The secretin-CCK test (18) was performed in the alcoholic group by giving a single intravenous bolus injection of secretin 1U/kg (Boots, England, lot 91.510/2), plus CCK 3U/kg (Boots, lot 91.295/2). The duodenal juice collected at three 15-minute periods was analysed for bicarbonate (19) and protein (20) concentrations after volume determination of each sample. Statistical analysis was carried out applying Student's t test, variance analysis, and the Sheffé test (21), with $p < 0.05$ as the level of significance. Results are expressed as mean ± SE. Prior to experiment, an informed consent was obtained from each patient.

Results. The alcoholic group was divided into two subgroups: a bicarbonate hypersecretor group (7 patients), with total duodenal bicarbonate output higher than 14 mmol/45 min, and a bicarbonate nonhypersecretor group (9 patients), with total bicarbonate output less than 14 mmol/45 min (9). On S-CCK test, in the hypersecretor group the means for total bicarbonate output, bicarbonate maximum concentration, and total volume were higher than means for the nonhypersecretor group. The total protein output of the hypersecretor group was also higher than that of the nonhypersecretor group (Table 1).

Differences in OGTT curves in the control and the alcoholic subgroups were not statistically significant (Fig. 1).

In the control group, the insulin curves at the 30-min and 120-min points were higher than the corresponding values in the hypersecretor and nonhypersecretor alcoholic groups. No difference was seen between the two alcoholic subgroups (Fig. 2).

No statistically significant difference was observed between the glucose levels in the control and alcoholic subgroups (Table 2). The peak and TIR values of both alcoholic subgroups were lower than those of the control group. No statistically significant difference was seen between the two alcoholic subgroups. Despite the absence of a statistically significant difference demonstrated by the variance analysis, the basal insulin level of the control group was marginally higher than that of the nonhypersecretor group—t crit = 2.14 and t cal = 2.82.

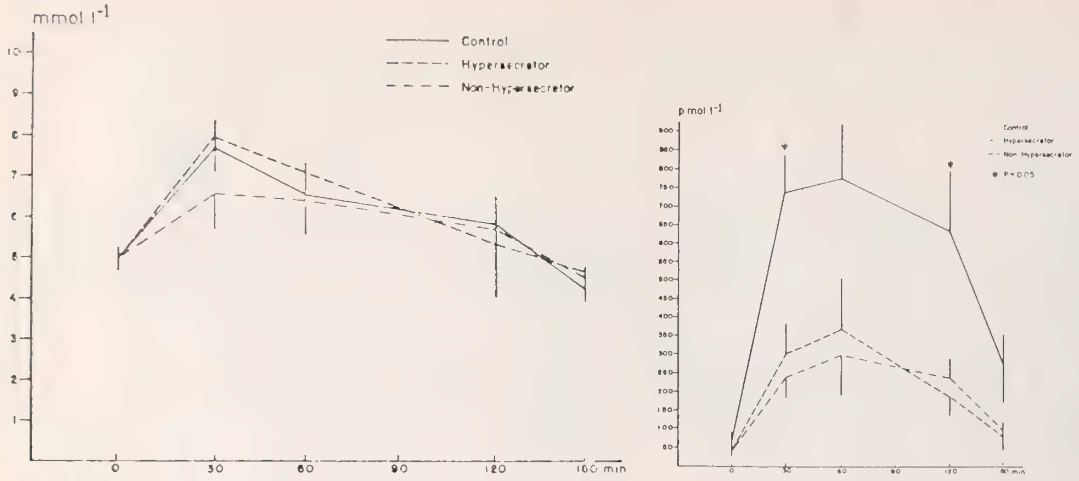

Fig. Left Blood glucose curves (mean ± SE). **Right** Plasma insulin curves (mean ± SE).

With respect to individual evaluation of participants in the alcoholic groups, we considered as normal a total HCO_3^- output less than 14 mmol/45 min, maximum HCO_3^- concentration higher than 66 mmol/L (9), and insulin TIR higher than 43 pmol × l^{-1} × 180 min^{-1}, this latter being the minimal value we found in the control group. In the hypersecretor alcoholic group, all patients had high HCO_3^- output and normal maximum bicarbonate concentration; for insulin TIR, 5 patients had low and 2 had normal values, as compared to the control group. In the nonhypersecretor alcoholic group, all with normal bicarbonate output, the maximal bicarbonate concentration was normal in 4 and less than 66 mmol/L in 5 patients; insulin TIR was normal in 4 and decreased in 5 patients; in 3 subjects (patients 8, 9, and 10), the decreased insulin TIR value was the only abnormal parameter, the maximum bicarbonate concentration and HCO_3^- output being within normal limits (Table 3).

There was a linear correlation coefficient ($p < 0.05$) between the insulin peak and the insulin TIR in the control and alcoholic groups. No correlation was found between bicarbonate output and insulin peak or insulin TIR in either alcoholic subgroup.

Discussion. Patients with chronic pancreatitis and marked pancreatic exocrine insufficiency usually have abnormalities of endocrine pancreatic function (22). In this paper, we are dealing with pancreatic endocrine function in alcoholics without an established exocrine hyposecretion secondary to chronic pancreatitis. We also assumed that exocrine pancreatic hypersecretion could be the first detectable functional alteration in patients during the early stage of alcoholic pancreatic disease (6, 9). In the present work, the total bicarbonate and protein output after hormonal stimulus showed the existence of pancreatic bicarbonate and protein hypersecretion, indicating pancreatic dysfunction, in 7 of 16 (40%) al-

TABLE 2

Blood Glucose and Insulin Responses in Controls and Alcoholic Study Groups (Mean ± SE)

	Control (C) (N = 8)	Alcoholic Secretors Hyper (H) (N = 7)	 Nonhyper (N) (N = 9)	Analysis of variance F*	Scheffé
Blood glucose					
Basal (mmol × l^{-1})	5.1 ± 0.3	5.2 ± 0.3	5.0 ± 0.3	0.0747 ($p > 0.05$)	NA
Peak rise (mmol × l^{-1})	7.9 ± 0.8	8.4 ± 1.0	7.4 ± 0.8	0.3314 ($p > 0.05$)	NA
TIR (mmol × l^{-1} × 180 min^{-1})	1077 ± 100	1107 ± 125	1044 ± 94	0.09 ($p > 0.05$)	NA
Insulin					
Basal (pmol × l^{-1})	64 ± 7	43 ± 7	36 ± 7	2.277 ($p > 0.05$)	NA
Peak rise (pmol × l^{-1})	1009 ± 156	442 ± 122	371 ± 97	7.80 ($p < 0.01$)	C > H = N
TIR (nmol × l^{-1} × 180 min^{-1})	103 ± 18	40 ± 11	39 ± 9	7.75 ($p < 0.01$)	C > H = N

* F crit (2, 21) = 3.47.

coholics studied, who had no previously diagnosed clinical alterations; these facts confirm former findings (9) that hypersecretion is a state which occurs in alcoholics without clinical symptoms of pancreatic disease.

The normosecretory behavior of the other nine alcoholics could be interpreted either as truly normal function or as a transition between the exocrine hypersecretory and hyposecretory states.

All the patients had normal glucose levels in OGTT by the criteria of the International Diabetes Data group (23). The basal, peak, and TIR glucose levels were similar in all patients; therefore, no endocrine dysfunction among the alcoholic subjects studied could be demonstrated by plasma glucose values.

On the other hand, the insulin secretion curve clearly showed a difference between controls and alcoholics (Fig. 2). The insulin peak and TIR (Table 2) were lower in both alcoholic subgroups than in the control group, and no difference was seen between the alcoholic subgroups. Ten of the alcoholics had low levels of insulin and normal glucose values in OGTT, indicating that insulin level is a more sensitive measure than glucose value in showing pancreatic dysfunction in established alcoholic chronic pancreatitis (22).

What is the significance of the decreased insulin response in both alcoholic groups, patients with and without bicarbonate and protein hypersecretion? At least two hypotheses may explain this. First, the decreased insulin production may be a sign of endocrine pancreatic dysfunction occurring together with or preceding exocrine pancreatic alteration (either a hypersecretory state or a decreased HCO_3^- maximum concentration). This idea is supported by the fact that low insulin levels were seen in both hypersecretor and nonhypersecretor pancreatic exocrine states. Second, there might be increased clearance of plasma insulin by the liver in alcoholics; against this hypothesis is the well-known fact that cirrhosis leads to hyperinsulinemia (24), and consequently the plasma of cirrhotics contains excessive levels of insulin, especially after meals (25).

In the hypersecretor group, in which the secretin-CCK test showed exocrine pancreatic hypersecretion of bicarbonate and protein, the insulin TIR was abnormally low in 70% of the patients. This means that some alcoholics with pancreatic hypersecretion had a normal insulin response.

In the alcoholic nonhypersecretor group, bicarbonate and protein output was not altered, but the maximum bicarbonate concentrations were low in 5 patients (56%), indicating pancreatic exocrine dysfunction; of these 5 alcoholics, 3 had normal insulin TIR. Altogether, insulin TIR was low in 5 patients (56%), and 3 of them had normal maximum bicarbonate concentration. Both maximum bicarbonate concentration and insulin TIR were altered in 8 of the 9 patients in this nonhypersecretory group.

TABLE 3

Evaluation of Participants in Alcoholic Subgroups

Subgroup & Pt. No.	HCO_3		Insulin TIR
	Max. conc.	Output	
Hypersecretor			
1	normal	↑	↓
2	normal	↑	↓
3	normal	↑	↓
4	normal	↑	↓
5	normal	↑	↓
6	normal	↑	normal
7	normal	↑	normal
Nonhypersecretor			
8	normal	normal	↓
9	normal	normal	↓
10	normal	normal	↓
11	↓	normal	normal
12	↓	normal	↓
13	normal	normal	normal
14	↓	normal	↓
15	↓	normal	normal
16	↓	normal	normal

These data indicate that in these alcoholics, who had no previous clinical abnormalities, the secretin-CCK test was diagnostic of pancreatic dysfunction in 75% of the patients, and the insulin plasmatic level was diagnostic in 55% of the patients. As the exocrine and endocrine alterations did not necessarily occur together, combining the secretin-CCK test and the insulin TIR was useful and revealed alterations in 93% of these alcoholics (15 of 16); the only patient with normal exocrine and endocrine response was a 29-year-old man who had the lowest alcohol intake of the group (100 gm/day for four years; patient 13, Table 3).

In our experience, the exocrine function test after hormonal stimulus was the most sensitive one for the diagnosis of pancreatic dysfunction due to alcoholism, but performing both tests substantially increased diagnostic accuracy.

In summary, our observations suggest that almost all chronic alcoholics without previous clinical alterations had exocrine pancreatic dysfunction and/or low plasmatic insulin levels in the absence of detectable alterations in OGTT.

Therefore, in order to diagnose pancreatic dysfunction in alcoholics accurately, both exocrine and endocrine studies should be performed.

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Reproductive Medicine Notes

Norbert Gleicher, M.D., editor

I will use this section to provide, in seminar format, updates on various areas of reproductive medicine. Since the majority of the readership of the Mount Sinai Journal of Medicine *comes from areas other than reproductive medicine, the major emphasis of these Notes will be the presentation of new developments in reproductive medicine to specialists in other areas of medicine. I hope that this will lead to the cross-fertilization of ideas between our field of reproductive medicine and other areas—NG*

The Cesarean-Section Epidemic

NORBERT GLEICHER, M.D.

In 1983 cesarean section became the most frequently performed surgical procedure in the United States. A total of 809,000 operative deliveries resulted in a national cesarean section rate of 20.4% (Ira M. Rutkow, M.D., Marlboro, New Jersey, personal communication). The continuous increase in national cesarean section rates was also reflected in a national sample of various cities, counties, and states which I first investigated in 1983 and which was reported in 1984 (1). At that point, 1982 and 1983 statistics for some of the national sites were not available. As the Table demonstrates, completed 1982 and 1983 statistics indicate a continuous increase in cesarean sections for the investigated sites. Preliminary 1984 data, both from the sample sites and from national statistics published by federal sources, suggest a further increase in the national cesarean section rate to above 22%.

I suggested in my earlier communication that the Consensus Development Conference on Cesarean Childbirth, which took place in 1982 (2), had failed—at least in the short term—to curb the continuous increase in national rates and argued that further action was needed to reverse the trend toward higher surgical delivery rates (1).

These conclusions were questioned by some authorities (3, 4) and supported by others (5). The resulting discussion, both in the literature and in more private professional circles, revived the issue of cesarean section as an important topic of critical discourse within our profession. Unfortunately, too much of the discussion has so far centered on the impact of the Consensus Development Conference and too little on the surgical rates themselves. With national cesarean section rates on a seemingly continuous upward course, a number of questions await satisfactory answers from the obstetrics community. Here are some:

What Is the Safest Mode of Delivery? An extensive review of the literature did not find this issue addressed until a very recent and rather controversial paper by Feldman and Freiman (6). Although their inappropriate presentation of maternal and fetal outcome statistics invalidated their conclusions, these authors have nevertheless to be credited with raising a very important question. At the recent World Congress for In Vitro Fertilization in Melbourne, worldwide statistics revealed that over 50% of pregnancies conceived in vitro have been delivered by cesarean section (7). The American literature has been abundant with reports about cesarean section deliveries of so-called "very valuable pregnancies"—pregnancies in patients with poor ob-

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TABLE

Cesarean Section Rates, Selected U.S. Cities, Counties, and States, 1977–1984

Area	1977	1978	1979	1980	1981	1982	1983	1984
Los Angeles County	15	16	16	17	17	18.3	—	—
California	15	16	16	17	17	18.4	—	—
Chicago	—	14	15	16	17	18	18.7	—
Illinois	—	14	15	16	17	18	18.7	—
New York City	—	19	20	20	21	22	20.3	—
New York State	14	16	17	18	19	20.7	20.8	—
Washington, D.C.	19	20	22	24	24	27	27.2	27.1
Philadelphia County	14	9	16	17	18	18.8	20.6	—
Pennsylvania	12	9	15	16	17	17.8	18.9	—
Greater Boston	19.5	20.8	22.4	22.1	22.6	20.3	20.3	—
Massachusetts	15.3	17.7	19.0	18.4	19.45	19.4	20.05	—
Wayne County (including Detroit)	—	—	—	9.9	10.8	11.4	12.3	—
Michigan	—	—	9.0	10.0	10.8	11.7	12.7	—
Virginia	—	—	9.3	9.3	13.7	15.0	15.9	—
St. Louis	—	12.9	13.2	13.4	13.9	16.3	16.76	—
Missouri	—	13.2	14.1	14.8	15.8	17.3	18.6	—
Atlanta	10.7	12.4	13.0	14.1	15.2	17.8	21.8	21.9
Georgia	13.2	15.6	13.5	15.5	15.9	19.3	19.6	21.1
TOTAL	14.8	15.0	15.6	16.0	16.9	18.2	19.0	

stetrical history or of advanced maternal age. Some textbooks, in fact, consider these situations an indication for cesarean section (8, 9). Are some babies more "valuable" than others? If delivery by cesarean section was in fact safer than vaginal delivery for patients of advanced age and poor obstetrical history, then surgical delivery should be safer for everybody. Does all of this indicate that we obstetricians consciously or subconsciously believe that cesarean section deliveries are "safer" than vaginal deliveries? If the answer is affirmative, who then can be surprised about cesarean section rates!

The safest mode of delivery obviously depends on maternal and fetal condition. Although a minority of superior institutions may have no maternal mortality with cesarean section delivery (10), in the nation as a whole, maternal mortality after cesarean section still significantly exceeds maternal mortality after vaginal delivery. More recent data also strongly suggest that short-term and long-term morbidity are increased after surgical delivery (11).

What Are Maternal Versus Fetal Rights? These issues do not enter into consideration when a cesarean section is performed for indications in the mother. However, when the surgery is performed to benefit the fetus, the question arises: At what point does the potential benefit to the fetus outweigh the risk to the mother? Feldman and Freiman (6) argue that the mother should be given the right to assume the risk of surgery on demand. If this right was accepted by our profession, we would set a surgical precedent which not even cosmetics surgeons are ready to concede to their patients.

Most obstetricians would clearly support the contention that the decision on cesarean section should be the physician's, based on sound medical judgement. Nevertheless, even the College in its commendable recommendations for vaginal delivery after previous cesarean section (12) concedes to the patient a veto right which goes beyond the usual informed consent prior to surgery. Acceptance of the patient's refusal of vaginal delivery after previous cesarean section and therefore repeating surgical delivery is considered good medical practice, even though innumerable studies have by now demonstrated the safety of properly selected vaginal deliveries after previous cesarean section (2). The patient's right to demand surgical delivery thus suggests (at least indirectly) that vaginal delivery may after all not be that safe. Furthermore, in the cesarean-section review process, "the patient insisted on it" is a nonrefutable vindication for every cesarean section.

What Is Proper Peer Review? Cesarean section is the only surgical procedure performed in accredited hospitals which is not subject to routine review by tissue committee. Recent regulatory changes have resulted in a clear decrease in the number of surgical procedures performed in the United States (13). Yet, as noted above, during the same period, cesarean section rates continued to increase. Whether this divergence is due to the disparity of the review process for cesarean section and other procedures is undetermined. The increase in cesarean section rates suggests, however, that a uniform peer review process needs to be put in place. In principle, cesarean sections should be reviewed like other surgical procedures for indication as well as outcome. Since departments are the sum of their practicing physicians, peer review of individuals will automatically lead to departmental review.

What Rates Are Appropriate? Cesarean section rates vary widely—between individual practitioners, between institutions, between geographic areas. It has also been shown repeatedly that cesarean section rates do not necessarily correlate with the risk status of the population (1, 14). In fact, insurance status of the patient and ownership status of the hospital often predict the rate of cesarean section to a much larger extent (1) than does risk status of the patient.

What constitutes an appropriate rate of cesarean section for a particular population has never been defined. Such a definition, which obviously has to encompass a range and has to take into account continuous changes in practice patterns, would appear to be a principal responsibility of the obstetrical community. Regionalization of obstetric care and the establishment of perinatal networks throughout the nation have defined the level of obstetric practice for most hospitals. Properly designed population studies should therefore permit the establishment of proper ranges in rates of cesarean section for hospitals with various levels of practice. The establishment of appropriate cesarean section rates will facilitate the review of institutions and, it is to be hoped, terminate the large variations in cesarean section rates between hospitals with similar populations and levels of practice (15). The possibility of reviewing hospital practice patterns in a comparative fashion will in turn facilitate the process of peer review of individuals.

Financial and Legal Questions. These are only a few amongst the most urgent questions raised by the issue of cesarean section. Many more should be part of the ongoing discussion within the obstetric community. Medical care cannot be discussed today without consideration of its financial aspects. A whole variety of financial issues have been and should be raised in the debate on cesarean section (1). Similarly, cesarean section cannot be reviewed without considering the medicolegal issues that every obstetrician faces (16).

However, neither financial nor legal issues represent the driving force behind the increasing cesarean section rates; these issues, with many others, contribute only peripherally to national cesarean section rates. A financial incentive system geared toward vaginal delivery rather than cesarean section, and a solution of the national liability insurance crises would undoubtedly shave a few percentage points off national rates.

Open Debate and Consensus Needed. None of these changes would, however, significantly reverse the national trend in cesarean section rates. This national trend will only be reversed if a consensus develops within the obstetric community that national cesarean section rates are in fact too high. Such a consensus does not at present exist. In order to prevent further antagonism of the public, we have to openly address the cesarean section issue as a major issue within our profession.

Open discourse is not necessarily bad in such a framework. After all, it is scientific debate that usually leads to scientific progress. Why should the issue of cesarean section be treated differently?

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Students' Corner

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Highly Selective Vagotomy: History, Development, and Current Clinical Results

Michael J. Greenall, FRCS, and Michael G. W. Kettlewell, MChir, FRCS

For many years it was widely believed that preservation of antral function was contraindicated in the surgical treatment of peptic ulcer. As a result many surgeons preferred to resect the antrum in the course of surgery for duodenal ulcer, whereas others recommended that if it were retained the antrum should be denervated, and drained and remain in continuity with the acid stream. However, surgery for peptic ulceration was greatly modified by the observation that there is an increase in serum gastrin after all types of vagotomy and the realization that vagal release of gastrin is relatively unimportant in humans. These observations suggest that antral resection or denervation is unnecessary in the treatment of peptic ulcer and that antro-pyloroduodenal innervation can be preserved. This forms the basic concept of highly selective vagotomy. This operation allows controlled gastric emptying through an intact, innervated pylorus, thus reducing the side effects of gastric resection and vagotomy with drainage.

In spite of the theoretical advantages of highly selective vagotomy, it was feared that retention of an innervated antrum would lead to an inadequate reduction of acid output. Furthermore, it was felt that the absence of a drainage procedure would lead to stasis and secondary gastric ulceration.

Highly selective vagotomy has now been used for over a decade and many long-term results are available. This review aims to evaluate the present status of highly selective vagotomy and to compare it with traditional operations for peptic ulceration and its complications.

The ideal operation for peptic ulceration would cure all ulcers permanently, produce no side effects, and have no mortality. It would also eliminate any possibility of malignant change developing within the stomach or gastric remnant. By the late 1960s partial gastrectomy and vagotomy with antrectomy or drainage had been used with great success for many years and at that time there seemed to be little reason to alter these methods of treatment. However, most of the large number of reports on the results of various operations for peptic ulcer were of little value for accurate comparative appraisal; most of the studies were retrospective and there were doubts as to the comparability of the series on which they were based.

Among the few randomized trials conducted during this time was the Leeds-York trial (1). This study compared the results after truncal vagotomy and gastroenterostomy with those after truncal vagotomy and antrectomy and polya partial gastrectomy in patients with duodenal ulcer, the patients being blindly assessed for modified Visick classification (1) at a gastric follow-up clinic. Patients classified as Visick Grade 1 had a perfect result, whereas those in Grade 2 had occasional symptoms easily controlled by minimal adjustment to diet. Patients in Visick Grade 3 had moderate symptoms causing some discomfort but which could be easily controlled and did not interfere with their lifestyle or work. Visick Grade 4 included patients with recurrent ulcers and those whose symptoms were so severe as to interfere considerably with work or enjoyment of life and caused dissatisfaction with the result of the operation. Five to eight years after truncal

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vagotomy and gastroenterostomy only 44% of patients had a perfect clinical result (Visick Grade 1) and only 70% had good to excellent results (Visick Grades 1 and 2 combined). Nineteen percent of patients had a moderate result (Visick Grade 3) and 11% were outright failures, due to either recurrent ulceration or postvagotomy symptoms. The results of the other operations were not significantly better, and all these procedures were followed by a high incidence and variety of side effects, although these were often mild in degree.

Goligher et al (2) subsequently showed that the clinical results five to eight years after truncal vagotomy and pyloroplasty were slightly worse than following truncal vagotomy and gastroenterostomy, although these findings were not based on a prospective randomized trial. Following truncal vagotomy and pyloroplasty, 68% of patients had good to excellent results. Symptoms such as epigastric fullness, diarrhea, and flatulence caused 18% of patients to be classified as Visick Grade 3 while recurrent ulceration accounted for most of the failures in Visick Grade 4.

A similar study from Glasgow (3) found no significant difference in the overall clinical results between truncal vagotomy with gastroenterostomy and polya partial gastrectomy. More recent studies from the United States (4–6) have also shown that truncal vagotomy and drainage is inferior to both truncal vagotomy with antrectomy and partial gastrectomy in curing ulceration. These studies also demonstrate that the side effects following truncal vagotomy and drainage are almost as severe as for vagotomy with antrectomy or partial gastrectomy, especially for dumping and diarrhea.

Reasons for Failure of Standard Operations

Failures of surgery for duodenal ulceration are due either to recurrent ulceration or to side effects of the operations themselves. Recurrent ulcers develop in about 2% to 6% of patients after partial gastrectomy (1, 3, 6), but in only 1% of those treated by vagotomy with antrectomy (1, 4, 6–8). Following truncal or selective vagotomy with drainage, the overall recurrence rate is about 6% to 9%, although the incidence varies greatly from one series to another and is occasionally as high as 30% (9). Prospective randomized trials show that the recurrence rate after selective vagotomy with drainage is similar to that after truncal vagotomy and drainage (10–12). This finding is in contrast to earlier claims that the more precise dissection required for the selective technique would result in fewer incomplete vagotomies (13).

Recurrence depends mainly on the incidence of incomplete vagotomy. In some series this has been as high as 40%, although it is approximately 20% in the majority of studies (14). Much depends on the individual surgeon and the operative technique, rather than on the type of vagotomy employed (15).

Although many failures of duodenal ulcer surgery are due to recurrence, treatment with either cimetidine (16) or revision surgery (17) is usually successful in the management of such patients. However, a far more difficult problem is posed by the various postgastrectomy or postvagotomy symptoms, especially dumping and diarrhea. Side effects after vagotomy and drainage had been attributed to both the drainage procedure (18) and the vagotomy (19). However, by the end of the 1960s studies on fécal fat excretion in animals (20) and gastric emptying of liquids and solids after vagotomy and drainage in humans (21–23) had shown that drainage procedure was probably the most important factor. All the standard operations for duodenal ulcer destroy or at least severely impair the normal control of gastric emptying. Both partial gastrectomy and vagotomy with antrectomy remove the antro-pyloro-duodenal segment, with the result that both liquids and solids leave the stomach in an entirely uncontrolled manner. Even the pyloroplasty or gastroenterostomy employed in vagotomy with drainage produce major disturbances in gastric emptying. After vagotomy and drainage there is precipitate gastric emptying of liquids (21, 22) and solids (23, 24); this results in rapid intestinal transit with dumping and diarrhea in susceptible individuals.

The absence of an intact pyloric sphincter also allows excessive reflux of bile into the stomach, which may cause nausea, epigastric discomfort, bilious vomiting, gastric ulceration, and even malignant change (25–27). Any improvement in the surgical treatment of duodenal ulcer therefore would not only have to reduce the incidence of recurrence but would also have to eliminate the side effects seen so frequently after the standard operations.

Theoretical Basis of Highly Selective Vagotomy

History. The increasing appreciation that many of the side effects of gastric surgery are due to loss of control of gastric emptying resulted in attempts to preserve pyloric function in the course of surgery for peptic ulcer. Vagotomy confined to the parietal cell mass was first used in dogs by Harkins and Griffith in 1957 (28). They reported that gastric emptying was satisfactory and that the parietal cell mass was adequately denervated because postoperative insulin tests were either negative or weakly positive. Although Harkins and Griffith suggested that a clinical application of this technique was feasible, another decade passed before interest was shown. Amdrup and Griffith (29) subsequently confirmed these early findings but also recorded a 47% increase in 24-hour acid secretion from Heidenhain pouches in dogs after denervation of the parietal cell mass. They concluded that the operation would eliminate dumping but might be followed by high incidence of recurrent ulceration because of excessive gastrin release from the innervated antrum.

The first vagotomy limited to the parietal cell mass in humans was performed by Holle and Hart (30). They performed this operation on the basis of their experimental findings in dogs that the antral nerves of Latarjet mediate inhibition of gastric acid secretion (31). Unfortunately, they added a pyloroplasty or antrectomy and therefore lost the control of gastric emptying. Vagotomy confined to the parietal cell mass, without a drainage procedure, was first used in humans by Johnson and Wilkinson (32, 33) and Amdrup and Jensen (34) who independently reported their experience within a few months of each other. It was hoped that this new operation would allow controlled gastric emptying through a normally innervated, intact pylorus and would therefore overcome many of the side effects associated with gastric resection and vagotomy with drainage.

Nomenclature. Since its inception there has been much confusion over the name of this new operation. Johnston and Wilkinson called their operation "highly selective vagotomy" whereas Amdrup and Jensen chose the term "parietal cell vagotomy." Others have used such terms as "proximal gastric vagotomy," "superselective vagotomy," "supraselective vagotomy," and "secretory selective vagotomy." This confusion was partially resolved when Amdrup and Johnston (35) agreed that if the operative dissection is determined by those anatomical landmarks provided by the nerves of Latarjet, it should be called "highly selective vagotomy," whereas if the antrum is defined with a pH probe it should be known as "parietal cell vagotomy." These two techniques produce similar postoperative reductions in acid output; however, the more extensive distal dissection with the pH probe results in significantly higher serum gastrin levels one year after operation (36).

Highly selective vagotomy is based on three main hypotheses (37). These were contrary to standard surgical theory at the time that highly selective vagotomy was introduced, but with greater understanding of upper gastrointestinal physiology, their importance has become more widely accepted.

Hypothesis 1: "Side effects of gastric surgery will be reduced if controlled gastric emptying can be preserved through an intact pylorus. If this is to happen, the vagal nerve supply to the antrum must be preserved." (37).

Many surgeons had previously suggested that preservation of controlled gastric emptying through an intact pylorus might reduce the incidence of side effects after gastric surgery (38, 39, 40). Successful attempts have been made to preserve pyloric function during the course of gastrectomy (41, 42), but these techniques have the disadvantage that they inevitably reduce the size of the gastric reservoir. The alternative approach of selective vagotomy without drainage relied on the integrity of the pyloric nerve to ensure normally controlled gastric emptying (43). Such attempts were relatively unsuccessful, as more than 50% of patients developed postoperative gastric stasis (44). The poor results of selective vagotomy without drainage was due to failure of surgeons to appreciate the importance of antral innervation in regulating gastric emptying. This concept was emphasized by Carlson et al (45) when they demonstrated that antral contractions not only control the passage of solid material into the pylorus but propel it back into the stomach for further trituration, thus forming an "antral mill." Antral denervation weakens this muscular activity (46, 47) sufficiently to require a drainage procedure.

Hypothesis 2: "The antrum can be left innervated with impunity. Provided that it remains in the acid stream it will not release excessive amounts of gastrin" (37).

The concept of retaining antral innervation was contrary to standard surgical practice, mainly because of experimental evidence in dogs of vagal release of antral gastrin (48). Smithwick et al (49) therefore recommended antral resection when treating duodenal ulceration, whereas Nyhus et al (48) suggested that if the antrum was retained it should be denervated and drained and remain in the acid stream.

Hansky and Korman later demonstrated an increased gastrin response to a protein meal after all types of vagotomy (50). This surprising finding was initially attributed to loss of acid inhibition, but the important demonstration by Debas and his colleagues (51) that stimulation of gastrin release by food can be inhibited with atropine after truncal vagotomy suggests the existence of vagal inhibition. Highly selective vagotomy is itself followed by an increased gastrin response to a meal, although this response is significantly less than in patients after truncal vagotomy and drainage (52).

It is now believed that the effects of vagal stimulation on gastrin release are far more complex than one of simple stimulation. Vagal stimulation may exert an inhibitory influence, possibly by vagal release of an inhibitory hormone—vagogastrone (53). Vagal inhibition of gastrin release in experimental animals is abolished by truncal vagotomy (54) as well as by resection of the antrum and duodenal bulb (55). However, vagal inhibition is unaffected by extragastric (54) or highly selective vagotomy (56), suggesting that this inhibitory hormone is released by vagal stimulation and that it is derived, at least in part, from the duodenal bulb and antrum. Thompson et al's demonstration of a postoperative elevation of serum gastrin in patients after highly selective vagotomy also suggests that this inhibitor of gastrin release may be derived from the fundus of the stomach, as this is the only segment of the gut denervated by this technique (52).

Hypothesis 3: "A vagotomy which is confined to the parietal cell mass preserves protective and inhibitory mechanisms which are sacrificed by truncal vagotomy with a drainage procedure" (37).

It was also hoped that highly selective vagotomy would preserve those protective and inhibitory mechanisms mediated by the nerves of Latarjet (31) and hepatic and celiac vagal fibers (57). The cephalic phase of pancreatic secretion provides an alkaline environment rich in enzymes for the neutralization of food as it leaves the stomach (58, 59). Truncal vagotomy reduces bicarbonate secretion in dogs (60), but the pancreatic response to insulin hypoglycemia is maintained after highly selective vagotomy in humans (61, 62). The antrum may also protect against peptic ulceration because of its alkaline mucoid secretions. Vagal nerve stimulation increases production of these protective juices (63) while truncal vagotomy destroys this mechanism.

It is now believed that the antrum plays an essential integrating role in the function of the proximal stomach (64), gallbladder (65), and pancreas (66) by both neural reflexes and gastrin release. These pylorocholecystic and pyloropancreatic reflexes are destroyed by truncal or selective vagotomy, but are preserved after highly selective vagotomy (65).

Effects of Vagotomy on Acid Secretion and Gastrointestinal Function

Acid Secretion. Maintenance of antral innervation led to fears that highly selective vagotomy would be followed by inadequate reduction of acid output. These fears were partially allayed by the demonstration of an increased level of serum gastrin after all types of vagotomy, as described above (50). Early studies (24, 67–70) also showed that basal, maximal, and insulin-stimulated acid secretion are significantly reduced within the first three months after highly selective vagotomy. However, significant increases in acid output then occur during the next two years, and these changes are accompanied by positive insulin tests on 50% of patients (67, 68). However, when acid secretion tests are performed more than five years after surgery, there is still a significant reduction in acid output compared to preoperative levels. Between one and five years, there is little change in acid output (71–73), although Liavag and Roland (72) found an increase in basal output whereas Lyndon et al (73) demonstrated an increased insulin response. In spite of this, the number of patients with positive Hollander tests remains constant, and both basal and maximal outputs are not significantly different from that measured in patients five years after truncal or selective vagotomy with drainage (74).

Gastrointestinal Function. The hope that highly selective vagotomy would be followed by less disturbance to the alimentary tract than truncal or selective vagotomy with drainage is supported by much experimental evidence. With preservation of an innervated intact pylorus, the gastric emptying of liquids is less rapid after highly selective vagotomy than after truncal or selective vagotomy with drainage (75, 76). However it is probably slightly faster than before the operation (75). Gastric emptying of solids has been found to be normal after highly selective vagotomy (61), whereas some studies have shown the early phase of gastric emptying to be delayed (77, 78), possibly due to delayed transit across a denervated proximal stomach. After vagotomy and drainage, the pattern of gastric emptying of solids is more variable; some patients exhibit stasis, some show abnormally rapid emptying, and others are relatively normal (23, 77, 78).

However, direct comparisons of these emptying studies are open to criticism because of the various methods used to evaluate gastric emptying.

The preservation of extragastric innervation after highly selective vagotomy is followed by less disturbance to fecal fat excretion (79) and to both endocrine and exocrine pancreatic function than after truncal vagotomy (61, 62, 80). The postprandial concentration of bile acids and pancreatic enzymes in the small intestine are normal after highly selective vagotomy, whereas after truncal vagotomy and drainage they are decreased (61, 62).

All these factors may result in a better long-term nutritional state after highly selective vagotomy. Although insufficient time has elapsed for this to be fully assessed, early studies (81, 82) suggest greater weight gain after highly selective vagotomy than after selective vagotomy and drainage.

Clinical Results

Operative Morbidity and Mortality

As highly selective vagotomy involves neither resection nor anastomosis, there was the hope of lower operative mortality and morbidity than following the standard surgery for duodenal ulcer. In a collected review, Johnston (83) found that the mortality was only 0.3% in 5,539 patients with duodenal ulcers treated electively by highly selective vagotomy. Cardiopulmonary disease accounted for 9 of the 17 deaths and necrosis of the lesser curve or fundus caused 5 others. Postoperative stasis developed in 0.7% of patients, but only 0.1% required reexploration. Only 0.6% of patients had further surgery for persistent stasis in the months or years after surgery.

The operative mortality and morbidity after highly selective vagotomy compare well with the standard operations for duodenal ulcer. Review of the literature for the past 10 to 20 years shows that the operative mortality of elective partial gastrectomy is at least 1.5%, although there have been reports of many hundreds of elective gastric resections without mortality (1, 84). The mortality of vagotomy and antrectomy is also about 1.0–1.5% and that of vagotomy and drainage is 0.6–0.8%, although there is some variation between different series (7).

Gastric stasis immediately after highly selective vagotomy is no greater than after vagotomy with drainage, and indeed stasis in a recent randomized trial was less than after truncal vagotomy and antrectomy (85).

Side Effects and Overall Visick Grading

Many prospective randomized trials have now compared side effects after highly selective vagotomy with those after truncal vagotomy and drainage and after vagotomy with antrectomy. In their trial of highly selective vagotomy against truncal vagotomy and pyloroplasty, Stoddard et al (86) found significantly less dumping, flatulence, bile vomiting, and postprandial distension after highly selective vagotomy. However, there was no difference in the incidence of diarrhea, nausea, heartburn, dysphagia, or food vomiting (Table 1). In their trials against selective vagotomy with pyloroplasty, both Kennedy et al (81) and Kronborg and Madsen (87) found significantly less dumping, diarrhea, and bilious vomiting after highly selective vagotomy (Table 2).

The overall results of highly selective vagotomy also appear to be better than after truncal vagotomy with antrectomy. Thus Dorricot et al (85) found significantly less diarrhea, vomiting, epigastric fullness, and heartburn after highly selective vagotomy, whereas Sawyers et al (8) recorded a lower incidence of dumping and diarrhea (Table 3).

In the Aarhus county trial, Amdrup et al (69) recorded significantly less dumping after highly selective vagotomy alone than after highly selective vagotomy with drainage or after selective vagotomy with either pyloroplasty or antrectomy. However, this study also showed that there was little difference in the incidence of diarrhea between the various operations.

The addition of a drainage procedure to highly selective vagotomy does not improve the overall clinical results and in Amdrup's study increased incidence of dumping significantly from 4% to 24% (69).

These figures suggest that the overall clinical results should be better after highly selective vagotomy than after the standard operations for duodenal ulcer. This is generally found to be true (Table 4), although there is some variation from center to center due to methods used for selecting patients for operative treatment, the criteria used to judge results, and differences in operative technique.

Recurrence

Many studies have now evaluated recurrent ulceration after highly selective vagotomy (Table 5). The overall recurrence rate 8 to 10 years after operation is about 8%. These results are similar to those after vagotomy with drainage, but are

TABLE 1
Results of a Prospective Trial of Truncal Vagotomy and Pyloroplasty (TV + P) and Highly Selective Vagotomy (HSV)

Symptom	HSV (n = 56) %	TV + P (n = 55) %
Early dumping	2*	16
Late dumping	4*	16
Flatulence	20*	42
Bile vomiting	4*	18
Postprandial distension	21*	44
Diarrhea	7	13
Nausea	13	24
Heartburn	16	18
Dysphagia	5	5
Food vomiting	7	11

* Significant difference.

Adapted from Stoddard CJ, Vassilakis JS, Duthie HL. Highly selective vagotomy or truncal vagotomy and pyloroplasty for chronic duodenal ulceration: a randomized prospective clinical study. Br J Surg 1978, 65:793–796.

higher than after partial gastrectomy or truncal vagotomy with antrectomy. The overall 8% recurrence rate after highly selective vagotomy also conceals recurrence rates between 10% and 25% in other series. This high rate of recurrence is due mainly to deficiencies in operative technique, resulting in a high incidence of incomplete vagotomy. Kronborg and Madsen (87) recorded a 58% incomplete vagotomy rate in their early studies, and Liedberg and Oscarson (101) recorded that no less than 83% of their patients were insulin positive in the immediate postoperative period.

In these early studies there was a tendency to denervate only the distal 1 to 2 cm of esophagus. Using this technique, Kronborg and Madsen and Hallenbeck et al recorded a poor reduction in postoperative acid output and a high incidence of both incomplete vagotomy and recurrence. When they altered their method to denervate the distal 5 cm of esophagus, they noted an improved reduction in acid output, a lower incidence of incomplete vagotomy, and fewer recurrent ulcers (102, 103). The extent of the distal dissection is also important. Johnston et al (104) noted that when they left 10 cm rather than 7 cm of innervated distal antrum, there was a higher incidence of both incomplete vagotomy and recurrence.

An important question is whether patients with high levels of acid output can be adequately treated by highly selective vagotomy, or whether an antrectomy should be included in their surgical management. From studies in patients after truncal vagotomy and drainage, Kronborg (105) suggested that those with a preoperative maximal acid output greater than 45 mmol/hr should have an antrectomy in addition to their vagotomy. Robbs et al (106) advised antrectomy if preoperative maximal output is greater than 25 mmol/hr, but others such as Blackett and Johnston (92), Cowley et al (107), and Holst-Christensen et al (108) have been unable to correlate preoperative acid output with recurrent ulceration. Furthermore, Johnston et al (109) found that two to five years after highly selective vagotomy for duodenal ulcer, the clinical results are similar in patients with acid hypersecretion (greater than 50 mmol/hr in men, 40 mmol/hr in women) as in those with outputs below these levels. The percentage reduction in acid output in the two groups is also similar. In three prospective controlled trials against vagotomy with antrectomy (8, 85, 90), the overall clinical results are in favor of highly selective vagotomy. Al-

TABLE 2
Results of Prospective Trials of Selective Vagotomy with Drainage (SV + GE) and Highly Selective Vagotomy (HSV)

	Kennedy et al (81)		Kronborg and Madsen (87)	
Symptom	SV + GE (n = 50)	HSV (n = 50)	SV + P (n = 50)	HSV (n = 50)
Operative mortality (%)	0	0	0	0
Recurrent ulceration (%)	2	2	8	22
Dumping (%)	37*	8	30*	6
Diarrhea (%)	12*	4	20*	6
Bilious vomiting (%)	14*	2	14*	4
Weight gain (kg)	0.3	4.2	—	—

* Significant difference.

TABLE 3
Results of Prospective Trials of Truncal Vagotomy with Antrectomy (TV + A) and Highly Selective Vagotomy (HSV)

	Sawyers et al (8)		Dorricot et al (85)	
Symptom	HSV (n = 49) %	TV + A (n = 50) %	HSV (n = 82) %	TV + A (n = 78) %
Dumping	0*	22	2	9
Diarrhea	2*	18	5*	14
Vomiting	—	—	7*	19
Epigastric fullness	8	0	10*	36
Heartburn	0	4	10*	23
Abdominal pain	—	—	11	13
Dysphagia	—	—	0	4

* Significant difference.

TABLE 4
Percentage of Good Clinical Results (Visick 1 + 2) After Prospective Trials of Highly Selective Vagotomy for Duodenal Ulceration

Authors	Follow-Up		Highly Selective Vagotomy	Truncal Vagotomy + Drainage	Selective Vagotomy + Drainage	Vagotomy + Antrectomy
	No. of pts	Years				
Sawyers et al (8)	174	1–4	96	—	—	94
Kennedy et al (81)	99	1–4	96	—	76	—
Dorricot et al (85)	160	1+	82	—	—	56
Stoddard et al (86)	111	1–4	93	78	—	—
Kronborg and Madsen (87)	100	1–4	78	—	68	—
Faxen et al (88)	47	1–2	88	—	83	—
Fraser et al (89)	137	2–8	87	70	—	—
Jordan (90)	53	1–2	97	—	—	85
Koffman et al (91)	149	1–4	81	85	—	—

though recurrent ulceration is somewhat more common after highly selective vagotomy than after vagotomy and antrectomy, in the Birmingham study (85) the reoperation rate was the same because gastric retention was more common after vagotomy with antrectomy than after highly selective vagotomy.

Carcinoma of the Stomach

Malignant change in the stomach after vagotomy or within the gastric remnant after gastrectomy is now well recognized (25). However, the exact mechanism whereby such changes occur remains uncertain. A profuse bacterial overgrowth develops in the hypochlorohydric stomach, which may produce carcinogenic nitrosamines from ingested secondary amines, nitrites, and nitrates (110). Alternatively, excessive bile reflux may also lead to gastritis, increased mitotic rate, and metaplasia. This may lead to malignant change after both partial gastrectomy and vagotomy with drainage (25–27, 111).

TABLE 5
Incidence of Recurrent Ulceration After Highly Selective Vagotomy

Authors	Follow-Up		Recurrence %
	No. of pts	Years	
Kennedy et al (81)	50	1–4	2
Fraser et al (89)	68	2–8	2
Blackett and Johnston (92)	233	5–12	11
De Miguel (93)	143	5–9	10
Goligher et al (94)	117	5–8	4
Grassi et al (95)	259	1–7	2
Jensen and Amdrup (96)	100	5–8	9
Liavag and Roland (97)	182	5–7	10
Madsen and Kronborg (98)	50	5–8	26
Storey et al (99)	93	5–9	16
Wastell et al (100)	52	3–7	6

It was hoped there would be a reduced incidence of malignant change after highly selective vagotomy, as the pyloric sphincter would remain intact, thus minimizing bile reflux. However, Meikle et al (112) found a marked increase in both the extent and severity of proximal gastritis three months after both truncal vagotomy with pyloroplasty and highly selective vagotomy. In contrast, Keighley et al (113) found significantly less radiologic evidence of reflux after highly selective vagotomy than following truncal vagotomy with pyloroplasty or with antrectomy. They also found that the postoperative concentration of bilirubin in the gastric aspirate is significantly less than after highly selective vagotomy. Similarly, Dewar et al (114) demonstrated that although antral gastritis is as great after highly selective vagotomy as before, gastritis in the body of the stomach is significantly less than in patients treated by partial gastrectomy.

In summary, although there is evidence of decreased gastroduodenal reflux after highly selective vagotomy compared to vagotomy with drainage and gastric resection, it remains to be seen if preservation of an intact, innervated sphincter will be associated with a reduced incidence of gastric carcinoma in the long term.

When Used for Complications of Duodenal Ulcer

Pyloric Stenosis. The symptoms of pyloric stenosis are due not only to fibrosis but also to edema and spasm. For this reason Johnston et al (115) recommended that pyloric stenosis can be treated by highly selective vagotomy with dilatation of the stenosis. Dilatation is provided either by the index finger or by Hegars dilators introduced via a gastrotomy in the denervated part of the stomach. Satisfactory results were later reported from many centers (116–120), but more recently Blackett et al (121) have warned that the mortality of this procedure is 4%, that 9% of patients develop recurrent ulceration, and that 10% require further surgery for restenosis.

Hemorrhage. In a review of the surgical management of hematemesis, Alexander-Williams et al (122) recorded that the mortality was 15% after 1,088 gastrectomies and 8% after 513 truncal vagotomies combined with pyloroplasty and under-running of the bleeding ulcer. Furthermore, rebleeding occurred in 14% of patients after both operations. Truncal vagotomy and pyloroplasty with under-running of the bleeding vessel remains the most widely used operation for bleeding duodenal ulcer, but Johnston et al (115) recommend that under ideal circumstances highly selective vagotomy might be used to advantage. However, they suggest that truncal vagotomy with drainage should still be used if circumstances dictate that the operation should be completed as rapidly as possible in poor risk patients.

When Johnston (123) later described highly selective vagotomy with under-running of the ulcer in 26 patients with bleeding duodenal ulcer, he found no deaths and no patient requiring reoperation for rebleeding. The antro-pyloro-duodenal segment requires careful reconstruction after under-running of the bleeding point. Johnston recommends that this can be achieved by carefully suturing the edge of the mucosa along the line of the incision with fine continuous catgut and then adding a second layer of interrupted sero-muscular silk sutures.

Highly selective vagotomy can also be used to advantage in patients whose hemorrhage has been controlled with endoscopic electrocoagulation (124) or those who are bleeding chronically.

Perforation. With the increasing tendency to perform definitive surgery at the time of perforation, good results have been reported after highly selective vagotomy (115, 125, 126). However, this method of treatment has not been widely adopted and is only recommended in favorable operative circumstances for surgeons experienced with the technique.

When Used for Gastric Ulcer

The earlier success of truncal vagotomy with drainage for gastric ulcer (127) had indicated that if the ulcer would heal with a vagotomy, better overall clinical results might be obtained with highly selective vagotomy.

The early results of highly selective vagotomy for gastric ulcer were encouraging. Johnston et al (128) found that the initial postoperative progress was satisfactory for both gastric and combined ulceration. In a longer follow-up of 300 collected cases, Johnston (129) recorded a 1% mortality and a 4% recurrence rate three years after operation. Gastric retention had not been a problem, dumping was less common than after gastrectomy, and fecal fat output was similar to that in preoperative patients. Hedenstedt and Moberg (130) recorded no operative mortality among 21 patients treated by highly selective vagotomy, "mostly without pyloroplasty," and in a follow-up of up to 4 years, no case of recurrent ulceration or of gastric cancer was found. Muhe and Rosche (131) found an operative mortality of 1.9% in 103 patients with gastric ulcer treated by highly selective vagotomy. Two years later only 2 of 43 patients had recurrence, and 83% of the patients had good or excellent clinical results.

One of the arguments against highly selective vagotomy for gastric ulcer was that if partial gastrectomy gave such good results in up to 90% of patients, with a recurrence rate of only 2%, what would be the advantage of highly selective vagotomy? These criticisms have been partially answered by Duthie and Bransom in their prospective trial of Billroth 1 gastrectomy against highly selective vagotomy with excision of the ulcer (132). They found that after 4 years, the Visick grading of the two operations was similar. Although the recurrence rate was 7% after gastrectomy and 15% after highly selective vagotomy, there was no evidence to indicate that either operation had a distinct advantage. At the 8-year follow-up, Reid et al (133) recorded that although the recurrence rate was still lower after gastrectomy, the overall Visick grading was in favor of highly selective vagotomy. Recurrence rates in this study were surprisingly high—23% after highly selective vagotomy and 17% after Billroth 1 gastrectomy—possibly reflecting the influence of endoscopy in diagnosing recurrent ulcers.

Conclusions

Highly selective vagotomy has now been used for about 12 years. It has the distinction of being more thoroughly investigated than any other operation for peptic ulcer and has often suffered from the disadvantage of unfavorable comparison with retrospective reports on the more standard operations. In the majority of well-controlled prospective studies, it has provided better overall clinical results than vagotomy with either drainage or antrectomy. Where recurrence has been a problem, it has often been due to a high incidence of incomplete vagotomy. However, recurrence is more common than after partial gastrectomy or truncal vagotomy with antrectomy.

The stimulus of this new operation has provided not only an opportunity for much clinical investigation, but in addition has greatly increased knowledge of gastrointestinal physiology. Highly selective vagotomy remains a controversial operation, but it is this controversy which has led to such spirited surgical investigation in the past decade.

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Editorial Note

The article: "Update on Hypnotics for Insomnia in Clinical Practice: I. Hypnotics," by Geoffrey Angelo W. DiBella, M.D., published in February 1986 (vol. 53, no. 2), was peer reviewed and accepted before methaqualone, one of the 16 hypnotics agents it discusses, was moved from Schedule II to Schedule I. The article should have stated that change in status clearly.

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A Neglected Etiology: Atheromatous Embolism

To the editor: The *Journal's* recent review of a neglected disorder, vascular pancreatitis (1), itself neglected a vascular etiology, namely atheromatous embolism. This entity is illustrated in the following case.

A 64-year-old hypertensive, diabetic woman entered Rush-Presbyterian-St. Luke's Medical Center with crescendo angina and failure of the left side of the heart. Physical examination showed a blood pressure of 190/110, a fourth heart sound, and no other abnormal findings. Specifically, no retinal atheroemboli (Hollenhorst plaques), absent peripheral pulses, or lower extremity tissue damage was seen. Laboratory evaluation demonstrated moderate azotemia. Cardiac catheterization confirmed severe coronary stenoses. Bypass grafting was contemplated. A stroke led to neurologic deterioration and severe obtundation. Renal failure and death ultimately supervened.

Autopsy showed unexpected, florid acute pancreatitis of unknown cause. Microscopy clarified its etiology: fragments of atheroma that had spontaneously dislodged from aortic atherosclerotic plaques and traveled distally (antegrade) in the bloodstream, lodging where the caliber of vessels diminished to meet their size. Similar emboli were found in the kidneys and accounted for renal failure. Atheromatous emboli also had lodged in hepatic, splenic, adrenal, and uterine small arteries and/or arterioles. Widespread and severe atherosclerosis was blamed for old and fresh myocardial infarction. Brain examination was not permitted, so the relative contributions of fixed vascular disease and emboli could not be assessed apropos the stroke. Several emboli in various organs were too old to ascribe to cardiac catheterization-induced atheroembolization.

Literature review confirmed that such emboli have been implicated as a cause of acute pancreatitis for many years (2, 3), and that they may follow aortic manipulation and also may arise on their own (4). They may also travel retrograde to coronary ostia, and thence, moving antegrade, may cause myocardial infarction. When localized aortic or carotid atherosclerotic plaques are responsible, and are amenable to surgical correction, further embolization is prevented. Thus the entity is of more than academic interest. The question of optimal medical management remains unresolved.

Atheromatous embolism thus always deserves consideration with regard to vascular pancreatitis, and more generally in damage to any organ in patients with severe ulcerative atherosclerosis. Any aged patient is a candidate for this condition (5).

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Letters to the Editor: letters should be short and will be subject to editing. Objective, constructive criticism of published articles or discussions of questions of general interest in medicine are welcome. Double-space the text of the letter and any accompanying references or tables.

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Acquired Immune Deficiency Syndrome

Constantin A. Bona, Guest Editor

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THE MOUNT SINAI JOURNAL OF MEDICINE
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The Epidemiology of Human Retrovirus-Associated Illnesses

STANLEY H. WEISS, M.D., AND ROBERT J. BIGGAR, M.D.

The class of RNA viruses known as retroviruses were first described in animals early in this century. Despite extensive efforts, it was not until 1980 that the first human retrovirus was reported by Dr. Robert Gallo and his colleagues at the National Institutes of Health (1). With the methodology established, a second human agent of similar type was soon found (2). These retroviruses were labeled human T-lymphotropic retroviruses (HTLV) types I and II because of their T-lymphocyte tropism.

Also in the early 1980s the acquired immunodeficiency syndrome (AIDS) epidemic unfolded (3–6), and it was quickly appreciated that the pathobiology involved an enormous disruption of T-lymphocyte-mediated immunity. Because animal retroviruses, and feline leukemia virus in particular, were known to cause similar syndromes involving a collapse of cell-mediated immunity, investigators sought a human retrovirus as a cause for AIDS. In 1983, researchers at the Pasteur Institute under the direction of Dr. Luc Montagnier isolated a new retrovirus from a New York homosexual man with lymphadenopathy (7), labeling this agent the lymphadenopathy-associated virus (LAV), but they were unable to prove that this agent caused AIDS. Investigators working with Dr. Robert Gallo also isolated the same class of virus, which they labeled HTLV type III. In 1984, they were able to demonstrate conclusively through virologic and epidemiologic evidence that it was the cause of AIDS (8–13). This virus is now recognized to be only distantly related to HTLV-I and -II, and is increasingly being termed the human immunodeficiency virus (HIV) (14, 15).

"HIV," Nomenclature, and Strain Variation. However, the name HIV may be too vague to delineate it from other immunodeficiency-causing retroviruses that may be found in the future and may be simultaneously too specific, since HTLV-III variants (both natural and genetically engineered) as well as closely related viruses may not cause immunodeficiency (16).

The intent of the nomenclature committee was that the term HIV would refer to the generic virus class of HTLV-III/LAV, including agents such as the AIDS-related virus (17). However, the individual viral strains, as well as the specific viral reagents developed from isolates and analyses based on those reagents, are to continue to be referenced by the names as originally assigned. The impacts of strain variation and of the varying growth and purification methods used for each strain upon epidemiologic analyses have not been critically or systematically reviewed. Such methodologic differences have led to significantly discrepant reports of the same study (cf. 18, 19), and thus delineation of the specific reagents remains critical. HIV serologic testing still retains the flavor of research testing, and the methods should be explicitly stated, not treated as a routine clinical laboratory result.

HTLV-I and HTLV-II

The epidemiology of HTLV-I and -II is still masked by the difficulty of distinguishing antibody "positives" in serologic tests from nonspecific reactions to cellular proteins or perhaps other viruses. This has been particularly a problem among populations with a heavy parasitic disease burden, such as those in the tropics. Newer antibody assays are much improved, although they still imperfectly distinguish anti-

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From the Environmental Epidemiology Branch, National Cancer Institute, Landow Building, Room 3C25, Bethesda, Md 20892. Address reprint requests to Dr. Weiss.

bodies to HTLV-I from antibodies to HTLV-II (20, 21). Meanwhile, the initial trail-blazing serosurvey research about these viruses must be interpreted with caution in light of the recognition of a family of related human retroviruses.

HTLV-I: Prevalence. In general, the prevalence of antibodies against HTLV-I is high in southern Japan (and emigrants from this area) and the Caribbean, where approximately 5% to 10% of the population has antibody. Studies in Africa have yielded inconsistent results, from none to 4% of the populations reported to be seropositive for HTLV-I antibody. The prevalence of antibodies in the United States population is low (<1%), but may be slightly higher in the southern states and among blacks. Prevalence increases with age and may be somewhat more common in older women than men. Households including a person with adult T-cell lymphoma have a higher prevalence of virus. Sexual transmission is probable, but other routes cannot be excluded, since preadolescent children are also positive. Vertical transmission of HTLV-I may account for some of the cases. Molecular analyses of HTLV-I isolates from varying geographic regions indicate remarkable genomic conservation.

HTLV-I: Associated Clinical Illnesses. Clinically, several illness have been associated with HTLV-I and -II, but the pathogenesis of these illnesses is poorly understood. There is clearly an association between a class of human malignancy, adult T-cell leukemia-lymphoma (ATL), and HTLV-I (22). This malignancy was first recognized by Japanese clinicians as a separate entity prior to the association with HTLV-I, and it appears to occur in high frequency in the southern islands of Japan. More recently, HTLV-I-associated ATL has been found to represent a high proportion of the lymphomas seen in part of the Caribbean, and sporadic cases have been reported from many parts of the world. Although cases of ATL occur in the United States, they have so far been unusual. HTLV-I has also been associated with the rare tropical neurologic illness acute spastic hemi- or paraplegias and perhaps other neurologic problems (23, 24). It has been suggested that other lymphoreticular malignancies may be directly or indirectly associated with HTLV-I (25), and that seropositive persons may be at increased risk for infections (20, 21). Since clinical abnormalities remain rare among those with clear evidence of past HTLV-I infection, these leads require further investigation.

The features of ATL are clinical rather than histologic (26). Patients generally have involvement of the skin and may have a circulating leukemia as well. Evidence of a disorder of calcium, including hypercalcemia and lytic bone lesions (nonmalignant), is common. Although the malignant ATL cell in some individuals has been shown to have suppressor function, it stains as T4-positive. Patients with this condition do much more poorly than those with other types of lymphoma, often dying within a year of disease onset despite therapy. The clinical course is frequently fulminant. The association with HTLV-I is based on both a high prevalence of HTLV-I antibodies in patients (>90%, depending on the restrictiveness with which the definition is applied to the clinical criteria) and the extraordinary titers of this antibody found in such patients, which may be several logs higher than in healthy seropositive subjects, and clonal integration of HTLV-I provirus into the DNA of the malignant cells. HTLV-I is capable of transforming T-lymphocytes in vitro.

HTLV-II. HTLV-II has only been marginally studied. The first isolate of HTLV-II, "Mo," was isolated from a patient with hairy-cell leukemia of a very rare T-cell variety (less than 20 cases reported) (2). The second viral isolate of HTLV-II was obtained from a moribund parenteral drug abuser with *Pneumocystis carinii* pneumonia (27). A third isolate of HTLV-II has been reported from a hemophilia A patient with recurrent infections and pancytopenia (28). A fourth known isolate, from a man with atypical hairy-cell leukemia, as well as the recent description of HTLV-II serologic reactivity in a patient with T-cell leukemia, suggest that HTLV-II may be tumorigenic in humans (29, 30). However, no disease, malignant or otherwise, has been formally established as being related to this virus. In part, the disease association and natural history of HTLV-II remain difficult to establish because of the extensive serologic cross-reactivities between this agent and the apparently more common HTLV-I. The two agents share about 60% homology, which accounts for the cross-reactivity.

HTLV-I and -II: Prevalence in Drug Abusers. In New York City, a substantial proportion of parenteral drug abusers have been reported to have evidence of past HTLV-I and HTLV-II exposure (20, 21). In contrast, HTLV-I and HTLV-II are rare among drug abusers in the United Kingdom (31). Recent studies have revealed that the prevalence of HTLV-I/HTLV-II varies widely among drug abusers in the United States with geography, age, and race (SH Weiss et al, unpublished observations).

HIV (HTLV-III, LAV) and AIDS

History. The AIDS epidemic was first reported in 1981, although in retrospect there were obviously cases in the late 1970s (32). Recently, a virus related to HTLV-III was isolated from cryopreserved serum collected in 1976 in rural Zaire from a 26-year-old woman who had previously lived in Kinshasa (33). This woman died in 1978 of an illness suggestive of AIDS, and such cases may prove to be important clues to the origins of HIV.

From the beginning, the major risk group in the United States and Europe has been homosexual men, but it was quickly appreciated that intravenous drug abusers and hemophiliacs were other populations at risk. The number of AIDS cases during the early 1980s doubled nearly every six months. Although this rate of increase had slowed somewhat by 1986 (34), possibly partly due to underreporting, it is still doubling every year. By mid-1986 there were more than 24,000 cases and, barring the emergence of some effective form of therapy or prevention, it is anticipated that there will be over 250,000 cases by 1991.

Prior to identification of HIV, evidence that AIDS was caused by an infectious agent was already substantial. Studies showed evidence of spread within members of a homosexual community by person-to-person contact, and between homosexual communities. The first members to acquire AIDS in low-risk areas were those with sexual contact in high-risk areas. Studies of homosexuals with AIDS and men with characteristic immunologic abnormalities (low helper-to-suppressor T-cell ratios or low absolute T-cell counts) both showed that homosexual men who were most sexually active, particularly as passive (receptive) partners in anal intercourse, were at greatest risk. The occurrence of AIDS among drug users and hemophiliacs also suggested a transmissible agent, but there were plausible arguments that all the risk groups shared another risk factor, particularly antigen overload. The occurrence of AIDS among transfusion recipients, some exposed to only a single donor, provided substantial proof of a transmissible agent.

Confirmation of Cause. The isolation of HIV and confirmation that it was the cause of AIDS were major breakthroughs. HIV was repeatedly isolated from AIDS patients and persons at high risk of AIDS (8, 9). Cross-sectional studies showed that antibodies against this agent were more common in AIDS patients than controls. Furthermore, the variation in the proportion of risk group members who were seropositive for HIV correlated with known temporal and geographic trends of the epidemic (12). And antibodies were absent in historic sera from before the onset of the AIDS epidemic, even in risk-group members and persons with a variety of clinical illnesses that might be considered suggestive of AIDS, as well as from blood donors drawn from the general population (8, 12).

Two major observations solidified a causal relationship. First, in a study of transfusion-associated AIDS cases, it was repeatedly possible to find (in almost all instances) a seropositive donor if sera from all the donors to which the patients had been exposed were available (35). Second, in prospective studies of homosexual men and parenteral drug abusers, only those who had preexisting exposure, as demonstrated by antibodies, went on to develop the immunodeficiencies and clinical syndromes of AIDS (21, 36–38).

Pathobiology. The biology of the virus has been unravelled with incredible speed, and we now understand much of the pathobiology of the AIDS process in considerable detail.

The virus is clearly tropic for CD4/helper (T4 or Leu3-positive) lymphocytes (39), and much of the disruption of the immune system is related to the lytic destruction of this cell population by HIV. However, other cells can be infected; in particular, evidence is emerging that infected monocytes or macrophages may be important in transmitting the virus to the helper lymphocytes.

Following infection, HIV uses reverse transcriptase to create a DNA version of itself and can then integrate into the genome of the cell, termed the proviral form. Once integrated, it may be quiescent until the cell is activated by some exogenous signal, such as an antigen. As the cell replicates, virus production is increased and lysis can occur.

Complicating the picture, some infected cells apparently contain cytoplasmic HIV without exhibiting a cytopathic effect. And some cells with integrated proviral HIV have been demonstrated to divide (in vitro) without expressing HIV. These cells, if appropriately stimulated, will express HIV, thus providing a model for persistent latent infection.

On a larger scale, there is a gradual decline of the number of helper lymphocytes circulating and in lymph nodes and a development of immune impairment that takes several years (13, 40). Immunologic abnormalities have been clearly linked with HIV seropositivity (41, 42). About 90% of infected men who were HIV-antibody-positive at onset (date of infection unknown) developed an inverted helper-to-suppressor ratio within three years (43). Antibodies and suppressor cells may rise initially in response to the infection (44, 45), but these responses are apparently ineffective.

No factors have as yet been identified as clearly influencing the rate of decline in vivo, but since this rate appears to vary from individual to individual, such factors may indeed exist. Speculative candidates include host variations (such as HLA type), frequency of lymphocyte-activating events (such as infections and exposures to foreign antigens), re-exposures to HIV, and co-infections with other viruses.

Manifestations of HIV Infection

Opportunistic Infections. Since the helper cells play a critical role in normal immune function, orchestrating both suppressor-cell and antibody responses to antigens, loss of this population has a severe impact on immunity. At what point immunity to one or another opportunistic agents fails may depend upon the particular clones that are lost as well as overall functioning of the immune system, and illness will certainly vary with exposure to different opportunistic agents. *Pneumocystis carinii* pneumonia is the most common initial manifestation of AIDS, probably because it is a widespread commensural organism (which is normally easily controlled by helper-lymphocyte-mediated immunity).

Kaposi's Sarcoma. One of the commonest manifestations of AIDS is a cancer of unknown etiology, Kaposi's sarcoma. Described over a century ago, this sarcoma was almost never seen in persons less than 60 years old and was never common in the United States (3 cases per million per year in men) prior to the AIDS epidemic (46). The endemic African form (47) is not associated with HIV, in contrast to the epidemic disease (48, 49), although the histology of both clinical variants appears identical (50), both having spindle-shaped cells and a vascular proliferation. Clinically, however, the epidemic variant is far more aggressive, and lymph-node involvement may be extremely common.

Since 1980, the relative risk of this sarcoma among single young-adult men (a surrogate denominator for the male homosexual population) has risen as much as 2,500-fold. Kaposi's sarcoma as a manifestation of AIDS has primarily occurred among homosexuals rather than other risk-groups, implying the existence of some etiologic factor that is exceptionally increased in this group. For reasons not understood—and perhaps partly related to underreporting—a decline in frequency of reports of Kaposi's sarcoma, even among homosexual men, has been observed in recent years (51). Among persons with AIDS, clinical immunosuppression is more closely associated with opportunistic infection than Kaposi's sarcoma. A limited number of persons with Kaposi's sarcoma have had limited disease without any evidence of immunodeficiency over several years of observation.

One tempting hypothesis is that development of Kaposi's sarcoma may require co-infection with another infectious agent. The epidemiology of this hypothetical agent ("Agent K") can be inferred from current observations on Kaposi's sarcoma in comparison to AIDS and common infectious agents.

The epidemiology of an Agent K may vary considerably from HIV. For example, fecal-oral spread is common with hepatitis A but rarely, if ever, occurs with hepatitis B and HIV. Fecal-oral transmission would be consistent with the infrequent appearance of Kaposi's sarcoma in groups at risk for HIV due to parenteral exposure (drug abusers, hemophiliacs, transfusion recipients, health-care workers). Furthermore, changes in sexual behavior among gay men have led to a decline in hepatitis A transmission (52). If the latency between Agent K acquisition and disease expression were short, a decline in Kaposi's sarcoma would be predicted, and this prediction would be consistent with recent observational trends. Further analogy with hepatitis A would suggest a period of only brief antigenemia (and thus infectiousness) and point to materials such as feces, rather than blood, in searching for an Agent K. In light of the difficulty of isolating new agents from nonsterile biologic materials, such isolation attempts will prove difficult. Persons with HIV-negative immunodeficiency conditions, such as renal transplant recipients, are also at increased risk of Kaposi's sarcoma.

If there is a common pathogenic mechanism in the development of Kaposi's sarcoma, it is unclear why renal transplant recipients, but not HIV-infected drug abusers, might be frequently exposed to Agent K. One possibility would be that a substantial parenteral dose might be required for infection.

Nitrite inhalants have been shown to be mutagenic, and some investigators have found that homosexual men who develop Kaposi's sarcoma have a history of greater recreational exposure to nitrite inhalants than those who develop opportunistic infections (53). However, some other epidemiologic studies have failed to detect such an association (38).

Lymphomas. In addition to Kaposi's sarcoma, the incidence of non-Hodgkin's lymphoma appears to be increased (54–56). These are generally of the Burkitt-like and immunoblastic varieties, both B-cell in origin. Other tumors have been suggested to be more frequent as well, particularly Hodgkin's disease, but this is still under investigation (57). Why any of these tumors should be increased and the relationship between the T-lymphotropic HIV, the associated immunodeficiency, and tumor development is not understood. The absence of integrated HIV in such tumor tissues suggests an early or indirect role for HIV in the pathogenesis of these tumors.

Brain Infection. HIV is also now recognized to infect the brain (58, 59), although exactly which cells in the brain is not clear. This effect appears to be independent of an effect on the immune system, because neurologic syndromes may be part of primary infection, which when present is most often similar to mononucleosis in symptomatology (60). Some neural cells have T4 receptors, to which HIV can attach and infect the cell. Symptoms of neurologic involvement vary from none to extensive behavior changes and neurologic signs.

Risk of HIV Transmission

Not surprisingly, the epidemiology of HIV parallels that of AIDS, but has a longer and more varied natural history. The risk of being exposed or infected with HIV is most strongly dependent on the probability of contact with the virus (that is, with a person already infected) and on the efficiency of transmission for given types of exposure. Since few non-risk-group heterosexuals in the United States have so far been infected with HIV, it is not surprising that heterosexual spread has so far accounted for a minority of U.S. AIDS cases. However, heterosexual spread is likely to be an increasingly common mode of transmission in the coming years. Assessments of the relative efficiency of modes of transmission will depend upon specific studies and cannot be inferred from AIDS surveillance data alone.

Host factors, such as genetic factors or co-infection with other agents, might potentially alter individual susceptibility. Until an assay which reproducibly quantifies exposure dose is developed, the minimal HIV infectious dose will remain uncertain. For hepatitis B, some persons with low dose exposures appear to develop some antibodies without actual infection (61). A similar phenomenon with HIV has not yet been described, and all seropositive persons are presumed to be potentially infectious and at risk of developing frank AIDS. However, the lower viral isolation rates from HIV-seropositive hemophiliacs compared to gay men indicates that some biologic differences among HIV-seropositive subjects does exist.

One instance of female-to-male transmission (62), supported by restriction enzyme analysis of the viral isolates (63), has been reported. Neither person was a risk-group member, and continued sexual exposure of the man was interdicted with the discovery of the woman's seropositivity. Although the two viral isolates are quite similar, the woman has persistently abnormal immune function, whereas the man has remained well, has not developed HIV antibodies, and his subsequent cultures have so far been negative (Weiss et al, unpublished data). The reason for their differential response remains uncertain.

Little is yet known concerning the possible biologic impact of HIV strain differences or repetitive HIV exposure in relationship to transmission or disease outcome.

Natural History

Animal experiments and prospective epidemiologic studies have quantified many aspects of the natural history of HIV infection (13). The incubation period between exposure and specific antibody production is uncertain. In chimpanzees, animals make antibody within 3 to 12 weeks after parenteral exposure (64–66), the timing and titer being related to dose of exposure. In humans, following parenteral exposure from blood transfusion, antibody appears in 4 to 7 weeks, but little is known about the appearance of antibodies following sexual exposure (67) or low-dose parenteral exposure. Thus, antibodies probably appear in humans within a time frame similar to that in the animal model, but certainty on this point is confused by occasional reports of virus isolation in an at-risk person months, or even more than a year, before antibody appears.

The frequency of acute illness following HIV infection is unclear, but probably most illnesses are too mild to bring the patient to medical attention. Antibodies against HIV are generally not detectable by current methodologies at the start of illness, appearing from one to three weeks after illness onset (68, 69, 69a). Symptoms, which generally last about 10 to 14 days, may include fever, fatigue, pharyngitis (sometimes ulcerative), myalgia, arthralgias, rash, and headache (68, 69a, 70). Lymphadenopathy is not uncommon and is variable in extent and timing, being both an early and a persistent abnormality in some persons (67).

There are few reports of AIDS in adults known to be infected less than a year, but children appear to run an accelerated course. The observed mean latency between AIDS development and the index transfusion exposure as a newborn is 1.2 years (71). The epidemic occurrence of AIDS, however, biases such surveillance tabulations toward those occurring earliest and thus deflates the true mean. Among adults, the observed interval between an index transfusion and AIDS outcome has been about three years. However, a mathematical model which accounts for such surveillance biases yields a mean latency period in excess of five years (72).

Among adults from all AIDS-risk groups known to be seropositive at least three years (duration of infection prior to that time unknown), at least 15% to 20% have developed AIDS (38, 40), and there is no indication that the occurrence of AIDS has yet peaked (Fig.). Once AIDS has been diagnosed, the average survival is about 1 year, with more than 80% dead within three years (51). Among seropositive homosexual men with absolute helper-cell counts initially below 300/dL, at least 40% were diagnosed with frank AIDS within three years (52, 73).

Risk Groups

The development of a national surveillance network contributed not only to greater understanding of the clinical dimensions of AIDS, but also to the recognition of several groups that were at increased risk of developing AIDS. Initially and still today, in the United States homosexual men constitute the greatest percentage of the patients (70%–75%), but intravenous drug abusers are also involved (17% heterosexual, plus 7% typically tabulated as among the homosexual men). In 1982, the first cases were reported among hemophiliacs, especially those treated for their coagulation disorder with factor VIII concentrate (74). In addition, Haitian and African patients with diseases that fit the strict Centers for Disease Control AIDS profile began to be observed both in the United States (75) and Europe (76). Investigations in Haiti (77) and the central African nations of Rwanda and Zaire (78, 79) were able to confirm that patients indigenous to those countries could be found in local hospitals and that the immune deficiency in those patients closely resembled the profile seen in AIDS patients in the United States. Furthermore, although incidence data were weak, it appeared from the timing of cases in Africans referred to Europe that AIDS was new to both areas (80). In addition, it was also soon observed that AIDS was appearing in subjects who had been transfused with blood or blood products in earlier years.

Whereas healthy risk-group members from regions with a high incidence of AIDS exhibit a high prevalence of HIV antibodies (12), within the U.S. relatively few healthy Haitians in these same regions and times have HIV antibodies (17, 81). Within the United States, the proportion of AIDS cases occurring among immigrants from "countries where heterosexual HIV transmission is believed primary" has decreased over time. One possibility is that the initial Haitian AIDS cases were related to infection before immigration to the U.S., and that slow secondary spread to their contacts occurred. Several studies have recently suggested a role for heterosexual transmission within the United States itself, and the CDC has further reclassified AIDS cases among such persons (for example, Haitians) in the "heterosexual spread" hierarchical category.

Homosexual Men. Among homosexual men, the overwhelming risk factors for being seropositive are the number of partners and the frequency of anal-receptive intercourse (36, 37). In areas distant from communities in which HIV infection is frequent, number of partners from the endemic areas supplants total number of partners as being the important risk factor (36). Other measures that correlate with these factors, such as nitrite inhalant use and fisting (the act of inserting the hand into the anus), will also correlate with seropositivity, because they are surrogate measures of anal intercourse. Some homosexual men who deny engaging in anal intercourse have been seropositive and developed AIDS, although the risk appears relatively low (82).

The implication of these data is that HIV is spread between homosexual men by exposure to semen, particularly through anal intercourse. This is biologically plausible, since the virus has been isolated from semen (83, 84). The degree of risk following oral exposure to HIV-containing semen is difficult to estimate because subjects in most studies performed both oral and anal inter-

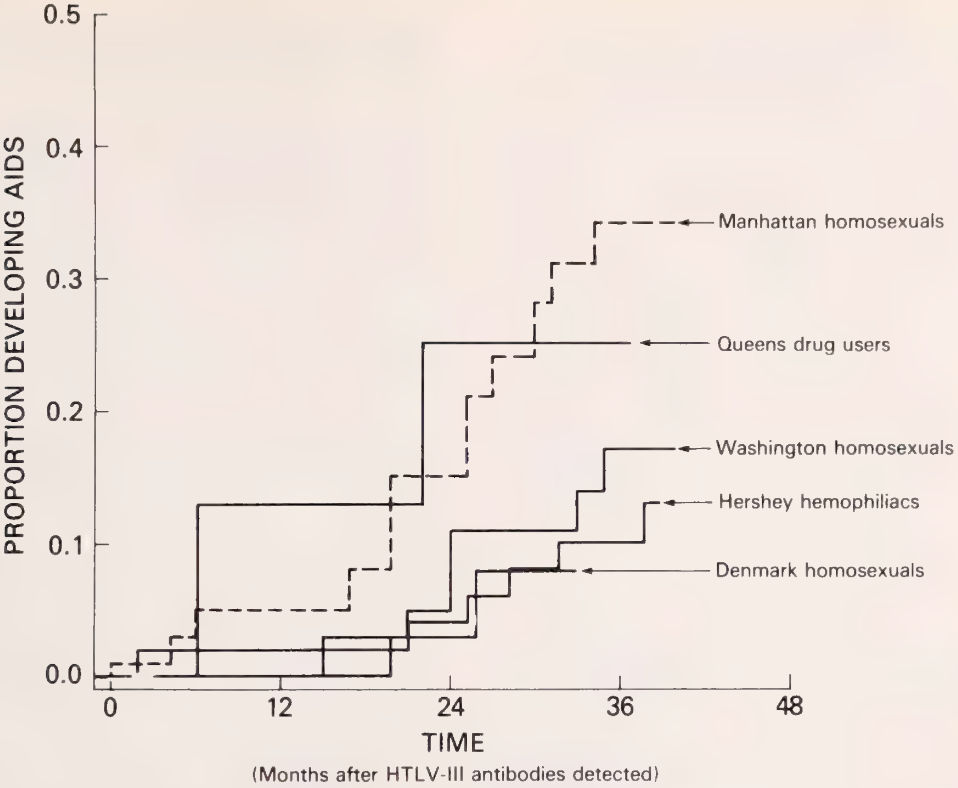

Fig. Development of frank AIDS among seropositive risk-group members followed up for three years. Actuarial incidence of AIDS among all 276 HIV-seropositive study subjects in five different cohorts of risk group members. Data computed by the Kaplan-Meier survival technique, with first seropositive specimen (or September 1982 for hemophiliacs with positive historic specimens) set as time 0. Adapted from JJ Goedert et al. (38).

course. The relationship between exposure to feces and risk for HIV transmission remains unclear (37, 38, 85, 86).

Following exposure, there are no factors established as influencing progression to immunodeficiency except duration of infection, but the studies examining this question have been based on relatively small numbers of seropositive men. Likewise, factors which influence which clinical manifestation of AIDS will occur first are unknown.

Parenteral Drug Abusers. Among intravenous drug abusers, use of shared, contaminated needles is presumed to be the major risk factor. The degree of risk with needle sharing depends upon the type of exposure. Hardly any health-care workers stuck with needles used on AIDS and HIV-positive subjects have become infected with HIV (62). However, drug addicts have vastly greater exposure to shared needles and may further enhance their risk by the practice of "booting," in which blood is drawn back into the syringe in order to extract any remaining drug in the needle or syringe. Under some conditions, a very small amount of blood left in a needle can transmit HIV (86a).

The variation in seropositivity between communities of drug addicts in different cities is huge—from less than 1% to over 50% of all addicts in treatment programs, those in New York City and adjacent communities in New Jersey being highest (87, and SH Weiss, unpublished data). The variation in seroprevalence by community argues that risk is related directly to some aspect of needle sharing within a drug community with a high HIV prevalence, since exposure to other possible risk factors, such as prostitution to support their drug habit, is probably similar in different communities. The contribution of heterosexual transmission to the spread of HIV infection among drug abusers requires further study.

Hemophiliacs. The dominant risk factor among hemophiliacs is exposure to commercially produced factor VIII concentrate (88–90). Cryoprecipitate concentrate is produced by processing the plasma of several thousand donors in batches, and it seems clear that the concentration procedure also can concentrate HIV. Retrospective studies have shown that hemophiliacs began to seroconvert as early as 1979, and that the seroconversion rate increased rapidly in 1981 and 1982 (91, 92), to the extent that by 1986 at least 70% of severe hemophiliacs treated during this period with commercial factor VIII concentrate are now seropositive (89). Even among the more mild hemophiliacs, one third are seropositive. Some countries produce their own factor VIII concentrate, and in one study, investigators estimated that as few as one or two infectious donors to the entire pool resulted in a batch of factor VIII concentrate that infected half of the recipients (93). Factor VIII produced commercially in Europe is often made from plasma collected in the United States and therefore shared the same infectiousness as factor VIII made in the United States.

Initially, it was hoped that the reactivity seen in hemophiliacs might represent immunization with noninfectious parts of the virus, or that lack of co-factors such as venereal and other infections might limit the risk of developing AIDS in this subgroup. However, adjusted for time of exposure, so far, hemophiliacs have followed the same progression to immunodeficiency and AIDS as other risk groups (38; also, see Fig.). Prior to the AIDS epidemic, a method of heat-treating the factor products to inactivate hepatitis viruses was developed; this procedure also appears to inactivate HIV and has been available commercially since 1985. Since the heat-treatment also inactivates some of the coagulation efficiency of the products, these products are more expensive. The certainty of HIV inactivation has recently been questioned (94).

Heterosexual Transmission. In the United States and Europe, there is evidence for some degree of heterosexual transmission. The plausibility of such infections has been demonstrated by animal models as well as in the women infected by insemination from a seropositive donor (95). In humans, about 10% of the wives of seropositive hemophiliacs have themselves been seropositive in preliminary studies (96, 97), presumably as a result of sexual exposure, since they are the only family members who are seropositive. The observation time is limited, and this relatively low transmission rate is best interpreted as a conservative lower limit which further confirms heterosexual transmission within the United States, rather than as an estimate of ultimate rates of transmission.

Female prostitutes in some cities have an increased prevalence of antibodies (98, 99), but such women often have other exposures, particularly intravenous drug abuse, that make interpretation difficult. Still, more than 200 women have developed AIDS whose only admitted risk factors have been heterosexual intercourse with men who were risk-group members (51). The biological plausibility of female-to-male transmission was enhanced by the demonstration that HIV can be isolated from cervical secretions (100, 101). The risk of female-to-male transmission is thought to be low, but there are at least 43 AIDS cases among men whose only admitted exposure was sex acts with a female risk-group member or prostitute (51, 98). A female health-care worker (who acquired her infection from a needle-stick exposure) was reported to have transmitted HIV to her seronegative, healthy long-term male partner (62). Restriction endonuclease mapping of the viral isolates from this pair confirms heterosexual transmission (63, and G Shaw and SH Weiss, unpublished data).

In Central Africa and Haiti, heterosexual transmission appears to be the major route of exposure (80). Other routes of exposure, including homosexual sex acts, needle exposure, transfusions, and "vectors," do not explain the seroepidemiology now being seen in these regions (80). However, as HIV infection becomes common, other routes of transmission in Africa, including blood transfusion and perinatal exposure, will increase.

Maternal-Infant Transmission. Infants born to mothers who are seropositive are at increased risk of infection (71, 102, 103), as are second children born to mothers who have already had a child with AIDS (71). The point at which infection occurs is not established, but in the majority it is probably in utero. However, one instance of postnatal infection, probably through breast milk, has been reported (104).

Occupational and Casual Transmission. Two simultaneous reports demonstrated no serologic evidence of HIV infection among health-care and research workers with exposure to AIDS patients and HIV materials (12, 105). These provided early, convincing evidence against significant risk from routine, casual exposure. These studies also indicated that the interim precautions suggested by the U.S. Public Health Service (106) and infectious disease experts (107), which were based on analogy to hepatitis B, appeared adequate.

A case report from England first demonstrated the possibility of HIV transmission to health workers by needlestick (108). The first U.S. study demonstrating transmission to a health-care worker by needlestick injury (62) indicated the need to teach and follow the promulgated guidelines rigorously. Other similar case reports have since been published (109, 110). These studies indicated that needlestick injuries among health-care workers were common (62) and potentially avoidable (111), but policies aimed at injury reduction have so far proven unsuccessful (112).

Approximately 2,500 health-care and laboratory workers have so far been enrolled in prospective studies specifically designed to examine the risk of HIV transmission in the workplace (Table). Among persons enrolled in these studies, only 2 instances of occupational transmission were clearly implicated (Table). These studies provide an indication of the magnitude of risk, since the study populations are clearly delineated. Furthermore, they provide the necessary background upon which to assess sporadic case reports. Similarly, in U.S. and European studies encompassing about 500 family members of patients with AIDS, related conditions, or asymptomatic HIV infection, there was no evidence of casual transmission. Based on these occupational and household studies of a total of 3,000 persons in whom no instance of casual transmission was documented, the statistical estimate of the upper limit of risk of casual transmission is approximately 0.1%.

Three case reports raise the possibility of casual transmission of HIV, even if it is rare. In one case, AIDS developed in a woman without known risk factors who provided home health care to a neighbor who died of AIDS (116). This care involved frequent and prolonged contact with his secretions and excretions, and she recalled having both some small cuts on her hands and an exacerbation of chronic eczema. A second, well-documented case of HIV seroconversion occurred in a mother who provided nursing care to her HIV-infected baby (117). In this instance, the care involved extensive unprotected exposure to the child's blood and body excretions and secretions. In the third case, an older sibling of a child who died of AIDS has been found to be HIV-seropositive following a bite (that did not overtly break the skin) as the only noteworthy potential route of exposure (118). In light of the many persons now infected with HIV worldwide, these rare case reports are certainly consistent with the above summary of study findings and indicate that casual transmission is remarkably rare in comparison to sexual and parenteral modes.

TABLE

Summary of Prospective Studies of Occupational HIV Transmission

		No. of subjects		
Ref. no.	Date published	Total	Non-risk-group*	Non-risk-group seropositives attributable to occupational exposure
12	Jan '85	188	~ 185	0
105	Jan '85	85	~ 80	0
62	Oct '85	359†	336	1†‡
113	Dec '85	151	~ 145	0
111	Apr '86	451	~ 425	1§
114	May '86	531	~ 500	0
115	Oct '86	300	~ 225	0
Unpublished studies and continued accrual in above studies		~650	~ 600	0
Totals:			~2500	2

* Estimated when not specified.

† Two seropositive non-risk-group members ("Health worker B" and "Health worker C") were excluded from tabulations. Both were referred for HIV testing because of immune abnormalities, and had histories of needlestick injuries. In addition, heterosexual transmission could not be excluded for Health worker B because her partner (no known risk factors after several interviews) was HIV positive upon referral.

‡ One seropositive woman, without known risk factors or needlestick exposure ("Health worker D"), was incompletely investigated and was therefore excluded from the tabulation.

§ One seropositive subject had been previously reported in greater detail as "Health worker B" in reference 62. See note † above.

Summary

The emerging understanding of the biology of both HIV and other human retroviruses permit hope that effective therapy and a vaccine will be developed. In the meantime, control measures will have to depend on our appreciation of the natural history of HIV through epidemiologic studies and on the ability to recognize who is at risk. Thus, these data will guide prevention efforts. The high risk of progression to overt illness that has been observed in HIV-infected risk group members (73) may provide impetus to initiate therapy trials before clinical end-stage immunodeficiency. The already enormous public health burden and its associated costs can be expected to continue to grow for the foreseeable future (13). We have only just begun to feel the social impact, and future worldwide repercussions on health care, social behavior, and society in general. A continuing, coordinated effort will be essential to cope with the scourge of HIV.

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Heterosexual Transmission of Human T Lymphotropic Virus Type III: Syphilis Revisited

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When AIDS was first recognized in 1981, public health authorities realized that this new and mysterious syndrome was unique. An arbitrary case definition was established by the Centers for Disease Control to allow the systematic collection of epidemiologic data, and to facilitate the identification of both the cause and the methods of transmission of this deadly syndrome. Today, AIDS is no longer a mystery. Science has identified the etiology: AIDS is an infectious disease caused by a retrovirus, human T lymphotropic virus type III (HTLV-III/LAV) (1).

Unfortunately, the original case definition identified only the late clinical stages of the disease, and thereby severely underrepresented the magnitude of the problem. Infection with HTLV-III/LAV causes a spectrum of illness ranging from an acute retroviral syndrome, to chronic lymphadenopathy, to subclinical T-cell deficiency, to mucocutaneous T-cell deficiency, to systemic T-cell deficiency, each of which can be etiologically linked to the virus (2). The critical pathogenic event in HIV infection is the progressive destruction of the T helper cell (3). The clinical presentation of the patient depends on the quantitative integrity of the T helper cell population (4).

The natural history of HTLV-III/LAV from infection to the development of AIDS is the consequence of a slow, chronic, progressive immunologic disease requiring perhaps 5–10 years to cause death. The cases of AIDS seen today represent unrecognized infections which were clinically silent for nearly a decade.

Our original understanding of the methods of transmission of HTLV-III/LAV was incomplete, once again hampered by focusing on AIDS. Scientific investigations have unequivocally documented the following methods of transmission of HTLV-III/LAV: bidirectional sexual transmission (male to male, male to female, and female to male); parenteral transmission (blood and blood products, sharing drug paraphernalia); and perinatal transmission (transplacental and postnatal breast milk). My intent here is to outline the scientific evidence for heterosexual transmission of HTLV-III/LAV, to focus on the magnitude of this new sexually transmitted disease in 1986/7, and to outline a reasonable public health approach in the twentieth century. More importantly, though, I hope to stimulate my medical colleagues to accept the responsibility for leading the battle to control the spread of this deadly sexually transmitted viral plague.

Evidence for Heterosexual Transmission of HTLV-III/LAV

The understanding of the epidemiology of HTLV-III/LAV infection is limited by focusing on AIDS. In light of our current understanding of the prolonged asymptomatic period of infection, focusing on AIDS cases allows accurate estimation only of the historical epidemiology of viral transmission 5 to 10 years ago. Likewise, focusing on the epidemiology of AIDS in terms of risk-group categorization continues to limit our understanding of the epidemiology of viral transmission. The actual magnitude of the problem today can only be determined by focusing on the extent of HTLV-III/LAV infection, not AIDS, in 1986.

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Originally, the purpose of defining risk groups for AIDS was to facilitate an accurate definition of the methods of transmission of AIDS. AIDS was well recognized in homosexual and bisexual men, intravenous drug users, and blood product recipients. Unfortunately, once several risk groups were recognized, a hierarchic categorization was established which actually hindered the recognition of the importance of heterosexual transmission. For example, if a person is an intravenous drug user and a prostitute, their risk of infection is classified as intravenous drug use, and their extensive sexual exposure is ignored. The underrecognition of the heterosexual population with AIDS is reenforced by the CDC surveillance report. As of August 4, 1986, 869 cases of heterosexually acquired AIDS—4% of the total cases (1% of male cases, 27% of female cases)—have been reported. However, the number of sexually active heterosexuals with AIDS reported to date, if one sorts by sexual preference and not by an arbitrary hierarchic categorization, is actually 26.5% of all cases.

Although evidence continues to document the parenteral and perinatal transmission of HTLV-III/LAV, sexual transmission is the most common method of spread of this virus today, and it will remain so in the future. Despite our current understanding of the epidemiology of the methods of transmission of this virus, many dangerous misconceptions remain. Many still believe this to be a "gay plague" or a virus which is only efficiently sexually transmitted by anal intercourse. Unfortunately this concept is in error. HTLV-III/LAV is an "old-fashioned" sexually transmitted disease, which depends on contact with the virus, independent of sexual preference.

Male-to-Female Transmission

Evidence which substantiates the efficient sexual transmission of HTLV-III/LAV from men to women is noted below.

1. Spouse Studies. Spouse studies continue to document male-to-female viral transmission. We at Walter Reed originally reported the frequent transmission of HTLV-III/LAV among the wives of patients with HTLV-III disease. Five of seven wives were infected with HTLV-III/LAV; their only risk was recurrent vaginal-genital, oral-genital, oral-oral contact with an infected male partner (5). Infection occurred in the absence of anal intercourse. An updated report documented that 15 of 34 women married to men with HTLV-III/LAV were also infected (6). Fishel et al. reported 14 of 24 women married to men with AIDS were infected (7). Salzman et al. reported that 17 of 48 (35%) female heterosexual recurrent partners of males with ARC/AIDS were infected (8).

2. Artificially Inseminated Women. Among 8 women who were artificially inseminated with HTLV-III/LAV-infected donor sperm, 4 became infected (9).

3. Intravaginal Infection of Chimpanzee. A 4-year-old chimpanzee was successfully infected when inoculated with HTLV-III/LAV intravaginally (10).

4. Prevalence in Prostitutes: Sexually Acquired? Seroprevalence studies continue to document increased infection rates in female prostitutes throughout the world (11–13). In the United States, infection of prostitutes is frequently attributed to needle abuse; however, the efficiency of transmission by sharing drug paraphernalia is unknown. The efficiency of semen deposited in the vaginal vault is noted above. The role of sexual acquisition in prostitutes should not be minimized. At Walter Reed, we have recently observed the rapid establishment of HTLV-III/LAV infection in an isolated group of young female prostitutes (prevalence rates greater than 5%) who were not intravenous drug users.

The data outlined above confirm the efficient sexual acquisition of HTLV-III/LAV by women through vaginal-penile sexual activity. Infection in women does not require the practice of receptive anal intercourse.

Female-to-Male Transmission

Evidence which substantiates sexual transmission of the virus from women to men is also scientifically well documented.

1. Sexual Contacts of Heterosexual Men. We reported the occurrence of HTLV-III disease in non-drug-using, strictly heterosexual men who had multiple sexual partners, or who had partners with multiple sexual partners (such as prostitutes) (14). It is important to emphasize that this study employed very active case detection methods. All patients (male or female) who came to Walter Reed Hospital in 1984 with signs and symptoms such as chronic lymphadenopathy, leukopenia, thrombocytopenia, hairy leukoplakia, oral candidiasis, chronic *Candida* vaginitis, recurrent or nonhealing skin lesions, prolonged constitutional symptoms, infectious diseases in which host immunity depends primarily on an intact cell-mediated immune response, or certain neoplastic processes were evaluated for retroviral infection. Fifteen of 41 patients with

HTLV-III disease acquired their infection through heterosexual contact. Sexual contact with an infected partner, having multiple sexual partners, or having sexual contact with partners who have multiple sexual partners (prostitutes) were the remarkable "risks" among these patients.

2. **African and Caribbean Data.** Observations in both Central Africa and the Caribbean heralded the reality of the sexual transmission of HTLV-III/LAV (15–18). The male-to-female ratio in these endemic areas approaches 1:1. Studies in Africa have documented the increased risk of HTLV-III disease in African men with multiple female sexual partners (19) and the increased risk of HTLV-III/LAV infection in African male customers of female prostitutes (11).

3. **Spouse Studies.** Spouse pair studies have also confirmed the efficient transmission of HTLV-III/LAV from women to men. We reported that 3/5 (60%) of husbands of women with transfusion-acquired AIDS were infected (6). Others reported data on the prospective acquisition of HTLV-III among spouses of AIDS patients, demonstrating that if the index case was the husband, 10/24 (42%) of the wives became infected, and that if the index case was a wife, 3/8 (36%) of the husbands became infected (7). These studies confirm the efficient female-to-male transmission of HTLV-III/LAV. Indeed, it would have been remarkable if HIV was the first example of a unidirectional sexually transmitted disease. Unfortunately, these data support the conclusion that HTLV-III/LAV, like all other sexually transmitted diseases, is efficiently transmitted bidirectionally.

Method and Efficiency of Heterosexual Transmission

The actual methods of heterosexual transmission and the relative efficiency of transmission by a single sexual contact with an infected partner, as well as the relative efficiency of viral transmission by different sexual activities, remain undefined. HTLV-III/LAV can be isolated from blood, saliva, semen, and vaginal-cervical secretions (20–25). Exchange of body secretions via genital-genital, oral-genital, oral-anal, or oral-oral contact may all play a role in viral transmission among sexually active heterosexuals.

Prevalence vs. Efficiency of Transmission. Because AIDS was originally recognized predominantly in homosexual men, a number of investigators concluded that HTLV-III/LAV could only be efficiently transmitted sexually by anal intercourse. It is true that the high seroprevalence of HIV in U.S. homosexual men makes the likelihood of a homosexual male coming into sexual contact with an infected partner greater than the likelihood of a heterosexual male coming into sexual contact with the virus. However, if a heterosexual male regularly employs prostitutes, this may not be true. In addition, a heterosexual man married to an HTLV-III/LAV-infected woman has an extremely high likelihood of coming into sexual contact with this virus. Caution should be employed not to interpret prevalence and efficiency of transmission as equivalent. The error is obvious.

It is not an unreasonable hypothesis that perinatal transmission of HTLV-III/LAV may represent the upper limit of human-to-human transmission of this agent. Perinatal transmission is an efficient method of HTLV-III/LAV transmission. It occurs approximately 40%–60% of the time (26–27). The actual efficiency of male-male transmission via anal intercourse remains undefined, although it is clearly efficient in light of the current HTLV-III/LAV seroprevalence data in homosexual men. However, it is unlikely to be more efficient than perinatal transmission.

The efficiency of male-to-female transmission has been documented in the setting of artificial insemination and recurrent sexual contact among spouses (5–9). In both settings the efficiency approaches 50%. In addition, recent studies noted above have documented that the efficiency of HTLV-III/LAV transmission among spouses is similar (47% and 43% respectively [6], and 42% and 38% respectively [7]), regardless of whether the husband or the wife was the index case.

A Dynamic Process. Infectivity of individuals with HTLV-III/LAV is likely to be dynamic: Infectivity is likely to vary among different individuals and within the same individual over time. It is noteworthy that spouses of hemophiliac males are less likely to be infected than their nonhemophiliac-spouse counterparts (28). The significance of these observations remains to be clarified; however, the concept of dynamic infectivity is important as one attempts to understand the apparent discrepancies in the frequency of sexual transmission when comparing one cluster to another.

HTLV-III/LAV Infection in 1986

Etiologic Diagnosis. Despite the universal availability of diagnostic testing of blood products for nearly 18 months, the medical and the public health community in the United States has continued to diagnose this infectious disease by arbitrary syndrome criteria, rather than utilizing the specific diagnostic test available to establish an etiologic diagnosis of a retroviral infection. The syndrome approach, while facilitating an accurate understanding of the epidemic a decade ago, fosters ignorance of epidemiology of the epidemic now. The diagnosis of HTLV-III/LAV infection is now possible and easily available. Use of diagnostic testing should be fostered by the medical community. The logic that resists the establishment of an etiologic diagnosis in clinical medicine or public health when available is unprecedented and should not be endorsed by the medical community.

The seroprevalence of HTLV-III/LAV has been documented in selected groups that include homosexual men (24% to 68% positive), intravenous drug users (25%–72% positive), and hemophilia patients (40% to 88% positive). Blood donors in the United States have a prevalence of 0.4/1000 (29). The limitation in studying each of these groups is that none of them reflect the current status of HTLV-III/LAV infection in young sexually active people.

Prevalence in Military Applicants. Recently the Department of Defense reported the results of the prevalence of HTLV-III/LAV infection in prospective U.S. military applicants (29). This population consists predominately of young adults in their late teens (54%) and early twenties (33% were 20–25 years old). Eighty-five percent were male and 77% were white. The overall prevalence of HIV in over 300,000 applicants tested was 1.5/1000. The prevalence among men was 1.6/1000 and among women 0.6/1000, a male-to-female ratio of 3:1. Seropositivity increased with age:

18 years old, 0.4/1000;
19 years old, 0.8/1000;
20 years old, 1.1/1000;
21–25 years old, 2.5/1000;
over 25 years, 4.4/1000.

Although the highest seroprevalence occurred among those over 25 years of age, the age of infection is often in the late teens or early twenties. Geographic areas in which AIDS has been epidemic for years had prevalence rates in this population of greater than 1.0%; for example, for applicants from New York City, the prevalence of HTLV-III/LAV infection was:

8/1000 for male applicants;
6/1000 for female applicants;
male-to-female ratio, 1.3:1.

Prevalence rates for applicants from the New York Metropolitan Area included Nassau 0.4%, Westchester 0.4%, Queens 0.6%, Kings 1.0%, the Bronx 1.3%, Manhattan 2.0%; and across the river in New Jersey, Union 0.5%, Passaic 0.5%, Monmouth 0.6%, Hudson 0.7%, and Essex 1.0% (30). These data illustrate that HTLV-III/LAV infection has spread to the youth of the New York Metropolitan Area. In 1986, deadly HTLV-III/LAV infection has become established as an endemic sexually transmitted virus in one of our nation's largest cities.

The Historical Perspective—Syphilis

Our society has no recent experience with a deadly epidemic, and so lacks a context within which to respond to one. Of all the epidemics of humankind, syphilis most closely mimics our current situation. Syphilis did not emerge gradually in the records of medicine but appeared suddenly at the end of the fifteenth century. Within a few years, it swept over the known world. Originally the respected scientists of the time attributed the cause to a recent astrological occurrence. Society responded with social and legal interventions based on mystery. Initially, syphilis was confronted by an attitude of fear and helplessness. However, over the next several centuries this attitude evolved into acceptance of the ravages of the great plague as part of life; scientific knowledge of its etiology and methods of transmission remained unavailable.

AIDS is no longer a mystery. Science has defined its etiology and major methods of transmission. Active intervention has occurred to minimize both transmission by blood and blood products (donor screening, self-donor deferral, and heat inactivation of factor VIII), and perinatal transmission (the recent recommendation of the CDC for routine prenatal HTLV-III/LAV screening for high-risk mothers).

Yet, despite these advances, it appears that little of this understanding has been incorporated into our medical and public health approach to the sexual epidemic. Why is society reluctant to apply aggressive intervention methods to curtail the sexual transmission of this plague? The obvious reason is that sexually transmitted diseases are difficult to control. Another reason is that skepticism and wishful thinking continue to be publicized by health care professionals in an attempt to reassure the public that the HTLV-III/LAV epidemic is under control. Overall, the rationale remains unclear. Yet these attitudes must be understood and overcome if scientific knowledge is to be utilized to challenge this virus, limit its ultimate impact on the world, and —finally—defeat it.

Public Health Approach: The Only Current Defense

Currently science cannot provide curative therapy or active immunization; however, science has provided us with the necessary tools to combat this plague and history provides us the methodology to control it. Our problems with controlling HTLV-III/LAV infection are not unique; our forefathers faced syphilis under similar conditions. In 1937 Thomas Parran, surgeon general of the U.S. Public Health Service, carefully outlined a platform to control the sexual plague of his time in his *Shadow on the Land—Syphilis.* He eloquently crystallizes what should guide us today to control an even greater plague, HTLV-III/LAV infection, using serologic antibody testing in just those ways Parran pleaded for the use of the Wassermann test:

> No matter how excellent the alibis for the little that we have done to control syphilis, nobody denies that it can be done. Great as are the unsolved scientific problems of syphilis, nevertheless we know enough now to save from the consequences of the disease both its victim and the society which now is burdened by him.
>
> The danger to one's self and others of harboring syphilis without knowing it could be averted if everyone were to submit voluntarily to a Wassermann test. Zealot though I may be concerning the advantages of universal blood testing, I realize it is not practicable to set up the machinery for tests on the whole population. The next best thing is to make a blood test whenever and wherever physical examinations are given, as routinely as the doctor now takes a pulse and blood pressure and listens to the heart action. Some venereal disease clinics make the Wassermann test a regular procedure; this should be performed on all patients with a venereal disease. Some hospitals make the Wassermann test a regular procedure in the admission of all patients for every case. All hospitals should do it. Every pregnancy means a Wassermann test is necessary. Certainly one place where there should be complete agreement as to the need for universal Wassermann tests, is in connection with applicants for marriage licenses.
>
> So many people are blind not only to the sufferings of others, but to their feelings of fear, of desperation, of blind panic when faced with a human tragedy such as syphilis can be and often is. We must be able to visualize the ascending death rate in terms of a man, a woman, a family. We must translate prevalence statistics into the terminology of pain. Then we can understand a falling death rate, a lessened prevalence, and a lower trend in the vernacular of healthy children, happy homes, and the continuity of family life.
>
> We are so apt to think we know nobody who conceivably might be suffering from syphilis. It is so easy to slip into the fallacy that this is the plague of the lower classes, the submerged tenth. It is so hard to imagine the situations in which it touches our own lives or may influence our future. Before we can generalize about syphilis or understand it in the mass, we must see it in the flesh and pity it in the misery of its victims.
>
> If you are the sort of good citizen who really is concerned about the suffering from syphilis, and irritated by the wastefulness of it, one of the greatest services you can render to your community—next to making sure that you are served by a well-trained, honest, full-time health officer—is in helping to shape and influence public opinion.
>
> If I have not done full justice through these preceding chapters to the event of syphilis control in our immediate past and present, it is because of this impatience with a day when we sit passive under the ravages of a preventable plague. Syphilis should be destroyed. If we are successful, the whole program will take a generation. I know we can do it if we are tenacious enough, if we fit our programs to the changing times, and if when we have made a good beginning, we do not in our complacency lose sight of a better tomorrow.

Physicians Must Lead the Battle

"Preventive medicine dreams of a time when there shall be no unnecessary suffering and no premature deaths; when the welfare of the people shall be our highest concern; when humanity and mercy shall replace greed and selfishness; and it dreams that all these things will be accomplished through the wisdom of man."—Milton J. Rosenau, MD, professor of preventive medicine and hygiene, University of North Carolina School of Medicine.

Most human infectious diseases become established long before scientific knowledge is available to alter the otherwise inevitable outcome. Rarely have we been given the opportunity to influence whether or not a disease will become endemic within the species. We have been given that rare and treasured opportunity to use our knowledge, to practice the art of public health, and to realize the dreams of preventive medicine. With that opportunity comes the burden of responsibility on each of us as physicians to use the available knowledge, to perform our public health obligations, and to facilitate the dreams of preventive medicine through human wisdom. Yet if we are going to take advantage of the opportunity that scientific knowledge provides us about AIDS, an option that fifteenth-century Europeans did not have when confronting syphilis, we need to incorporate knowledge of the past five centuries into our approach to this disease. As physicians we must remember: HIV is a sexually transmitted virus efficiently transmitted bidirectionally, no different from any other sexually transmitted disease except in that it is a deadly viral infection. Anyone with new sexual contacts is potentially at risk. The methodology to approach and control this sexually transmitted disease is available. The model of syphilis and the surgeon general of the Public Health Service in 1937 have provided us with a platform for action to combat this modern-day plague.

My intent here has been to focus on certain important observations which I myself have found helpful in both assessing the magnitude of our problem and anticipating the future of this epidemic. The reasons for my impatience with the present situation are, I hope, clear. We physicians can no longer sit passively under the ravages of a preventable plague. Our failures will be measured in terms of the death of our youth and their children; our success will go unnoticed. The window of opportunity has begun to close. We in the medical profession can and must take the lead in the control and eradication of HTLV-III/LAV. Make no mistake: we, through human wisdom, will defeat this virus—but we must act now.

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The Etiologic Agent of AIDS

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Abstract

A novel human retrovirus, LAV/HTLV-III, has been identified as the etiologic agent of the acquired immune deficiency syndrome (AIDS). It is a member of the lentivirus family and is characterized by helper/inducer lymphotropism, a cytopathic effect on infected T4 cells, a unique genomic organization with five open reading frames, and antigenic determinants not related to other human retroviruses. Its role in the etiology of AIDS has been strongly supported by its frequent isolation from all groups of patients with AIDS or at risk for AIDS, its biological properties, the finding of antibodies to it in persons with the disease and with AIDS-related complexes, and evidence of LAV/HTLV-III transmission in blood transfusion-associated AIDS patients. This discovery will undoubtedly facilitate the understanding of the pathogenesis of the disease, its prevention, and its treatment.

The acquired immunodeficiency syndrome (AIDS), first described in 1981 in the United States, is characterized by opportunistic infections and neoplasms, particularly Kaposi's sarcoma (1–3). In fact, most of these illnesses appear to be secondary to a profound depression of cellular immunity (4, 5). Preceding the diagnosis of AIDS, infected persons frequently have a history of early symptoms, which include unexplained fever, weight loss, diarrhea, persistent generalized lymphadenopathy (PGL) (6, 7) and helper T-cell depletion (8). These earliest clinical manifestations are often called AIDS-related complexes (ARC).

As this epidemic unfolded, certain groups became obvious as being at risk for AIDS. Initially homosexual men (9) and drug abusers (10) were identified as at high risk. Later, investigators recognized similar cases among Haitians (11), Africans (12), hemophiliacs (13), blood-transfusion recipients (14), and sexual contacts of AIDS patients. The incidence of the disease in these last three groups, as well as in intravenous-drug users, indicated that transmission of the disease was either by blood or by sexual routes, and strongly suggested the involvement of an infectious agent in the etiology of AIDS. In 1982, the concept of a virus as the cause of AIDS was already widespread. This assumption was supported by the observation that some hemophiliacs treated by blood products developed AIDS; the human plasma derivatives used to treat hemophiliacs are usually filtered through a membrane that eliminates contamination with bacteria and fungi.

At that time, almost all known viruses were etiologic candidates. But in 1983, new virologic results pointed to the possible role of a new human retrovirus in the etiology of AIDS (15–17). This new virus was named lymphadenopathy-associated virus (LAV) and, later, by others, human T lymphotropic virus type III (HTLV-III) or AIDS-related virus (ARV) (18, 19); most recently the term human immunodeficiency virus (HIV) has been proposed (19a).

Background on the Etiology of AIDS

A number of viruses, including cytomegalovirus (CMV), Epstein-Barr virus (EBV), and hepatitis B virus (HBV), have frequently been seen

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in patients with AIDS and thus have been suggested as causative agents.

CMV. CMV infections were known to affect the immune system (20, 21), and CMV particles were observed in Kaposi's sarcoma tumors (22). Based on previous reports on the association of CMV with Kaposi's sarcoma (23) and on a very high prevalence of active CMV excretions among homosexual men (24), CMV was for a while a leading candidate. However, a number of AIDS patients do not have CMV infections, and many healthy homosexuals—as well as nearly 100% of the general population in some areas of the world—are infected by CMV without obvious adverse consequences. These observations argued against the involvement of this virus in AIDS.

EBV. Reports on the unusually high frequency of infectious mononucleosis in some persons with AIDS-associated syndromes (25) and the incidence of lymphoproliferative diseases (Burkitt-like lymphoma or non-Hodgkin's lymphoma) in AIDS patients were consistent with the hypothesis that EBV may cause AIDS. The comparative level of EBV genome in AIDS patients and in patients with Burkitt's lymphoma or nasopharyngeal carcinoma (25) added support to the association of EBV with AIDS. But, despite these observations, EBV is known to be present in the vast majority of healthy individuals; it was therefore difficult to define the exact role of EBV in the onset of the disease.

HBV. From the beginning of the epidemic, it was observed that the distribution of AIDS and patterns of spread resemble those of hepatitis B virus infections. The role of HBV in the development of AIDS has frequently been postulated (26, 27) and has been supported by prospective studies showing a high incidence of HBV infections in homosexuals (26, 28) and a high prevalence of antibodies to HBV surface and core antigens in AIDS patients. However, the appearance of AIDS in sexually active homosexuals who received hepatitis B virus vaccine clearly indicated that HBV was not the primary cause of AIDS.

Retroviruses were also considered logical candidates because of their recognized ability to cause immune suppression and T-cell proliferation. Moreover, one of these viruses, feline leukemia virus (FelV) is known to cause an AIDS-like disease in domestic cats (29). At that time, the only recognized human retrovirus was human T-cell leukemia type I (HTLV-I), initially isolated in 1980 from patients with T-cell malignancies (30, 31). Several factors supported the possible role of this virus in AIDS. It is T lymphotropic, particularly for T4 cells (32), the population of lymphocytes affected in AIDS, and it seems to be transmitted by blood or intimate contact (32). Its endemic presence in Central Africa and in Haiti, two areas endemic for AIDS, also supported this hypothesis.

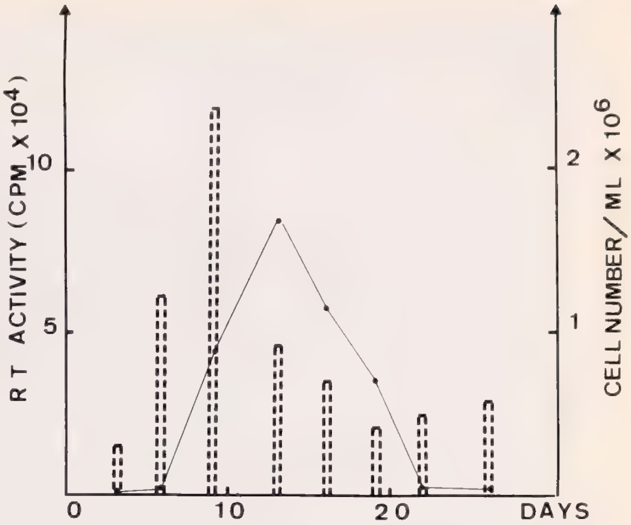


Fig. 1. Production of LAV/HTLV-III by primary T lymphocytes established in culture from an AIDS patient. Leukocytes were isolated from peripheral blood of an AIDS patient, stimulated with phytohemagglutinin (PHA-p) for 3 days, then continuously grown using interleukin-2. Reverse transcriptase (RT) activity in the culture fluids (—●——) and cell proliferation (– – – –) were measured twice a week.

However, several criteria required of the responsible agent of AIDS were not fulfilled by this virus:

- Only a few cases of AIDS or ARC have been reported in southwest Japan, where HTLV-I infections have a very high prevalence.
- Only a small number of AIDS patients have antibodies to HTLV-I gag proteins (33, 34), although a much higher percentage of positive cases for antibodies to membrane antigens of HTLV-I–infected cells has been reported (35).
- HTLV-I proviral DNA sequences were observed in only a few cases of AIDS (36).
- HTLV-I was only occasionally isolated from patients with AIDS or prodromes (37).

On the basis of these observations, HTLV-I was unlikely to be the etiologic agent of AIDS and is probably only another opportunistic pathogen.

Indeed, in 1983, the hypothesis of a retrovirus as the primary cause of AIDS was confirmed by the discovery of a new human retrovirus isolated from patients with AIDS and related syndromes (15–17). We refer to this unique virus here as LAV/HTLV-III, and we report here evidence linking it to the disease, as well as its biological and biochemical properties.

Isolation and Detection of LAV/HTLV-III

In 1983, the first LAV isolate was obtained in our laboratory from a homosexual male with lymphadenopathy syndrome (15). Since that time, similar viruses have been isolated by us (17, 38–40) and by others (18, 19, 41) from a large number of patients with AIDS or AIDS-related syndromes and from healthy persons at risk for the disease (homosexuals, hemophiliacs, intravenous-drug users, Zairians, Haitians, recipients of blood and of bone-marrow transplants). The incidence of virus isolation from patients who are seropositive for antibody to LAV proteins is very high (nearly 80%), except in AIDS patients at a very late stage of the disease.

Experiments to isolate the virus are usually performed using the patient's T lymphocytes from peripheral blood or from lymph nodes. Mitogen-stimulated T cells are cultured in the presence of interleukin-2 (IL-2) and antibodies to human α interferon, and the virus is detected by measuring the reverse-transcriptase activity in the cell-free supernatant. Virus production and expression can also be monitored by electron microscopy and by immunofluorescence, using a patient's serum containing antibodies to LAV proteins. The virus is generally detected in cell supernatant fluids within 2 weeks; virus production then decreases rapidly, and decline in cell proliferation (discussed below) follows (Fig. 1).

The virus has been isolated from mononuclear cells obtained from bone marrow (42), brain (43), semen (44, 45), and saliva (46). The presence of infectious virus has also been described in cell-free plasma from patients previously identified as virus carriers (38, 42).

General Characteristics of LAV/HTLV-III Isolates

LAV/HTLV-III is unique. Its morphologic appearance under the electron microscope is clearly distinct from the other two known human retroviruses, HTLV-I and HTLV-II. Immature particles bud at the cell surface, with a dense crescent in close contact with the plasma membrane, and mature particles typically have a small, dense, eccentric core (Fig. 2). During maturation, bar-shaped nucleoids can be observed in the viral particles. This structure is very close to that of lentiviruses, such as equine infectious anemia virus (47) or visna, and the resemblance was rapidly confirmed by the finding of other similarities in their biologic and biochemical properties.

Biologic Properties

In cell cultures, LAV/HTLV-III behaves differently from most other retroviruses, which usually replicate without appreciable cytopathic effect and often immortalize the cells. In contrast LAV is highly cytopathic. When normal T lymphocytes from a healthy donor are infected with LAV, virus replication occurs only after stimulation of the infected cells, and the release of virus particles is associated with the formation of many multinucleated giant cells (17) and the loss of viable cells (48) (Fig. 1). Furthermore, disappearance of the OKT4/Leu3a (helper/inducer) phenotype is related to the kinetics of virus production (49, 50).

Studies on fractionated T lymphocytes have clearly demonstrated, both in vivo and in vitro, the selective tropism of LAV for the helper/inducer subset of T cells (50), and the T4 molecule has been found to be associated with the receptor for the virus on the cell surface (51, 52).

Many of these biological properties of LAV correlate with the pathological picture of the disease observed in vivo.

However, the mechanism by which LAV induces a cytopathic effect on infected cells, particularly on the helper/inducer lymphocytes, is not yet known. The possible release of potent immunoregulatory factors by infected cells has been suggested (53).

In addition to fresh T_4 lymphocytes, other human T or pre-T cell lines (H9, HUT78, CEM-CCRF), as well as some B lymphoblastoid cell lines, were found to be susceptible to LAV infection (54–56). These cell lines have proved useful in producing large amounts of virus, since the cytopathic effect of LAV is less pronounced on some of these lines. The adaptation of LAV to B-lymphoid cells has also suggested that cells other than T lymphocytes might be infected in vivo and serve as a reservoir for the virus in vivo.

Biochemical Properties

Viral Proteins. Immunologic assays using the Western blot (Fig. 3) and radioimmunoprecipitation (Fig. 4) techniques have identified antigens of molecular weight 110,000, 68,000, 55,000, 41,000, 34,000, 25,000, 18,000 and 13,000 daltons in purified LAV as major targets of antibody re-

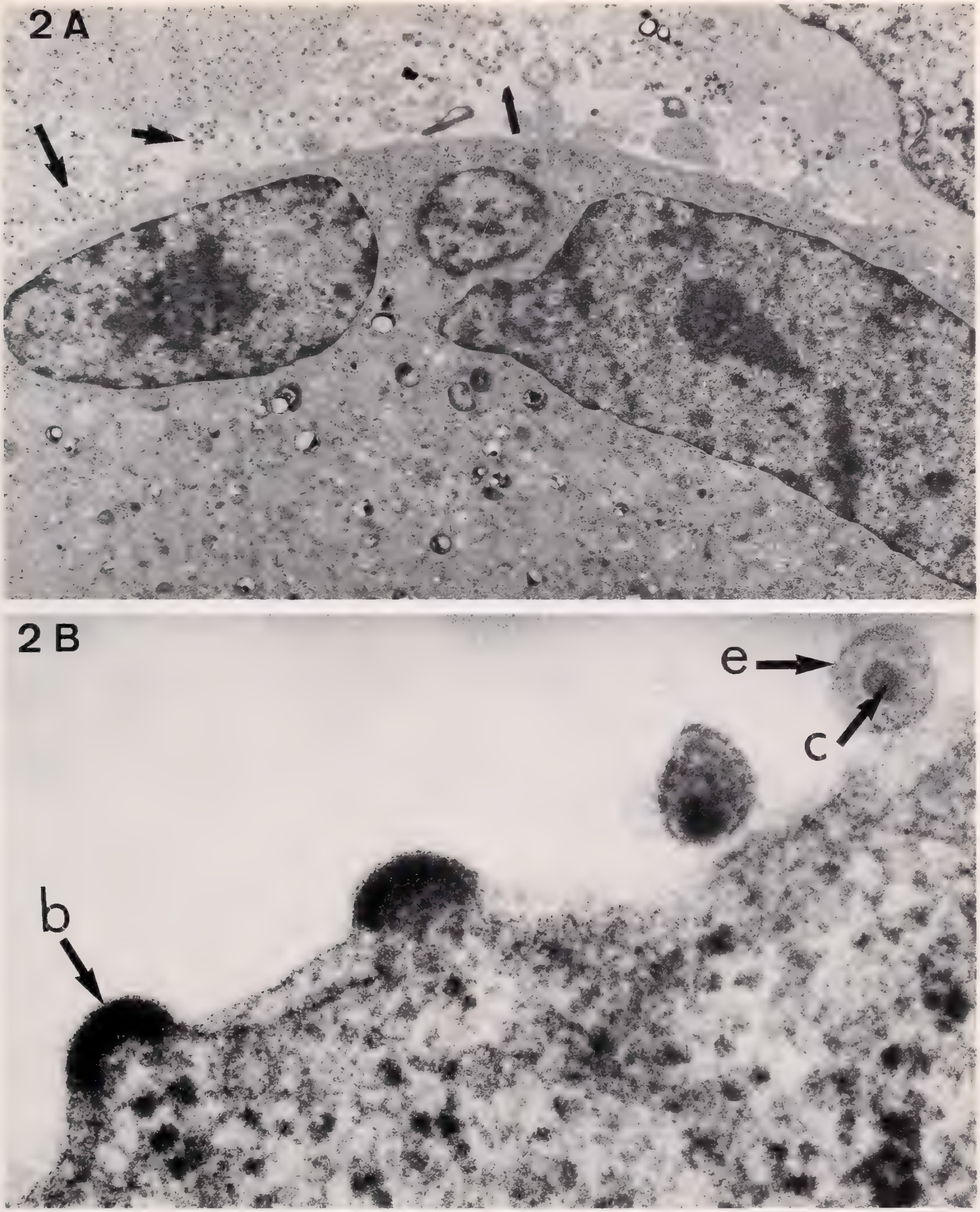
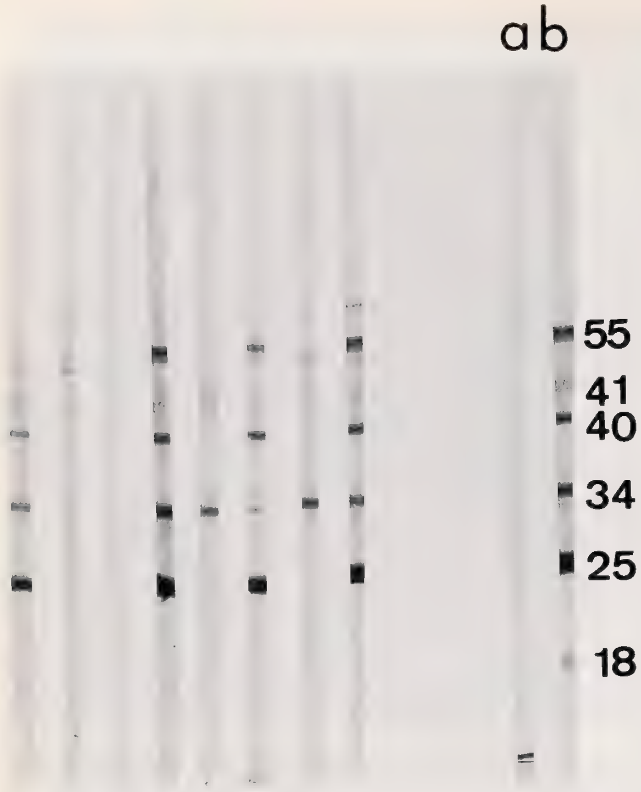


Fig. 2. Electron micrograph of LAV/HTLV-III-infected cells. **A** Ultrathin section of multinucleated infected T cell releasing numerous viral particles (arrows). **B** Budding particles and mature virions with typical small eccentric core; b, budding; c, core; e, envelope.

actions with sera of AIDS patients (17, 47, 57–59).

All these antigens are believed to correspond to the envelope glycoprotein and gag-pol proteins of the virus. Nucleotide sequence analysis, discussed below, has confirmed that p 25, p 18, and p 13 are gag gene products and p 55 is the gag precursor of these three proteins. Proteins of 110 K and 41 K have been characterized as membrane proteins. P 110 is glycosylated and represents the external glycoprotein (57, 60). P 41 is the transmembrane protein. These two proteins are pro-



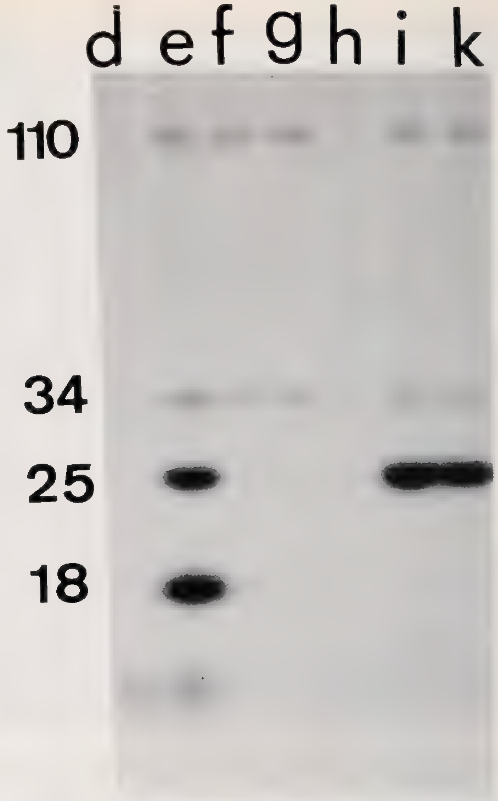


Fig. 3. Left Identification of LAV/HTLV-III antigens recognized by patients' sera. Proteins from purified, disrupted LAV/HTLV-III were separated by polyacrylamide gel electrophoresis, transferred to nitrocellulose sheet, and incubated with test sera. Antibody binding was revealed using a peroxydase enzymatic reaction. Strip a, negative control serum; strip b, positive control serum; other strips, sera from AIDS and ARC patients.

Fig. 4. Right Radioimmunoprecipitation assay (RIPA). Polyacrylamide gel electrophoresis of immune complexes between [^{35}S]cysteine-labeled virus and specific antibodies. Lymphadenopathy-AIDS-virus (LAV/HTLV-III) immunoprecipitated by normal serum (lane a), by LAV reference serum (lane b), by AIDS patient's serum (f, g, i, k), and by anti-human-T-cell leukemia virus (HTLV-I) (lane h). Numbers indicate level of molecular weight of markers.

teolytic cleavage products of an env gene precursor of molecular weight 160 K.

The antigenic properties of LAV have been compared with those of other animal and human retroviruses using radioimmunoprecipitation and competition radioimmunoassay techniques (17, 47, 61). The results have clearly demonstrated that LAV is a novel and unique retrovirus whose major core protein, p 25, is distinct from that of other retroviruses, including HTLV-I and HTLV-II. The only cross-immunoreactivity reported was with equine infectious anemia virus (47), which is a lentivirus.

Molecular Biology. As with all retroviruses, the genome of LAV consists of a high-molecular-weight RNA (60s–70s). This genome has been molecularly cloned and entirely sequenced (62–64). The genetic organization of LAV (Fig. 5) was deduced from the nucleotide sequence analysis and was found to be unique so far as is known among retroviruses. The overall length of LAV provirus is about 9.2 Kb; several open reading frames (orf) have been identified. The first, second, and fourth coding regions are the gag, pol, and env genes of LAV/HTLV-III. The env gene is exceptionally long and is separated from the pol gene by a third open reading frame termed Q (Sor or A by other authors). It has been suggested that this gene, previously unrecognized in other retroviral genomes such as HTLV-I and HTLV-II proviral DNAs, may play a role in the cytopathic effect of LAV (62), although its function is not yet known.

The env gene is followed by a fifth open reading frame, named F (also Lor or B by others), which extends into the U3 element of the long terminal repeat (LTR). This region was thought to encode for a trans-acting factor, but recent data indicate that a region located between Q and env genes is required for trans-activation (65, 66). Therefore, the exact role of the coding regions Q and F as well as the mechanism of trans-activation of the LTR promoter remain to be defined.

The genetic organization 5′LTR-gag-pol-orfQ-env-orf F/3′ LTR is conserved among the

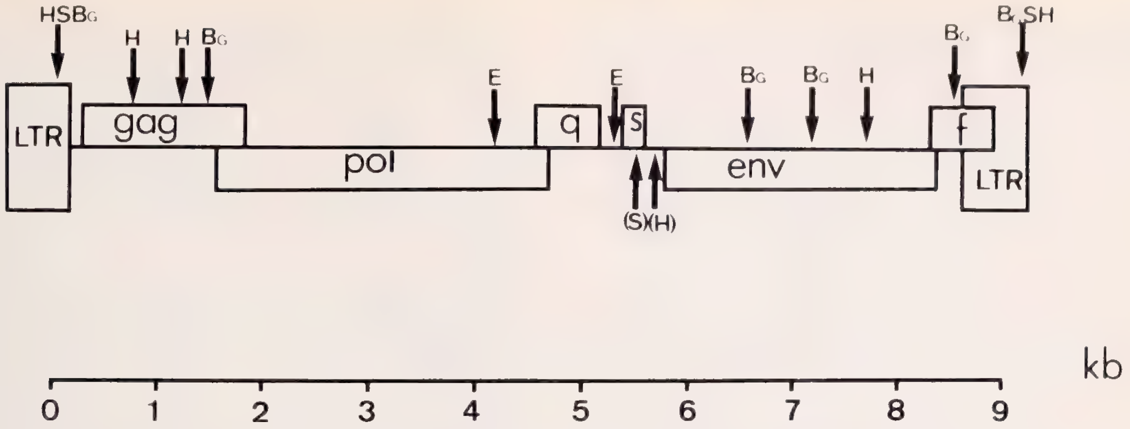


Fig. 5. Genetic organization of proviral DNA. LTR, large terminal repeat; gag, coding for core proteins; pol, coding for reverse transcriptase; env, coding for envelope glycoprotein; Q and F, coding for unknown products; S, transactivator region, also known as tat gene. Arrows indicate site of cleavage for some restriction enzyme.

different isolates of AIDS viruses. However, restriction enzyme analysis of different isolates obtained from individual patients has demonstrated genomic variations (42, 67). The most substantial divergences have been found in the part of the genome that encodes the envelope gene (67). This genomic heterogeneity of the AIDS viruses must be considered in the ongoing attempt to develop a vaccine against LAV/HTLV-III.

The availability of LAV/HTLV-III-cloned probe has also allowed the detection of the virus in fresh tissues from AIDS and ARC patients. Viral sequences were found mainly in lymph node, brain, and spleen (42), but only a very low number of cells express viral RNA (42). The discovery in 1985 of proviral DNA in cells from the brains of some AIDS cases (43) suggested a possible role of the virus in the central nervous system dysfunctions (dementia or encephalopathy) that have been observed in AIDS patients (68) since 1983.

The nucleotide sequence analysis of LAV/HTLV-III genome has also clearly demonstrated that this virus is a novel and distinct retrovirus, since no nucleic acid homology has been found with other known retroviruses except to some extent with visna virus (69).

LAV/HTLV-III as a Lentivirus

Many characteristics of LAV/HTLV-III (Table 1) have suggested resemblances between this virus and members of the lentivirus family, such as visna virus and equine infectious anemia virus. Like visna virus, LAV/HTLV-III seems to be involved in pathologic effects on the central nervous system in vivo and has cytopathic effects in vitro on infected cells. Other similarities between these viruses include their morphology in electron microscopy, their genome, and their envelope glycoprotein size. Data on the nucleotide sequence analysis of visna virus indicated a genomic organization close to LAV, with a central region between pol and env and a phylogenetic relationship between the reverse transcriptase and endonuclease sequences of visna and LAV/HTLV-III (70). This analysis confirms that LAV/HTLV-III is a member of the lentivirus group of retroviruses.

Seroepidemiologic Studies

Sera of a large number of patients with AIDS or associated symptoms, persons at risk for AIDS,

TABLE 1

Main Characteristics of the Etiologic Agent of AIDS

Morphology
Budding at the cell surface
Mature particles with dense, small eccentric core (41 nm)
Biology
T_4 lymphotropic
Cytopathic for T cells
Viral antigens
Internal proteins
  p 13, p 18, p 25
  precursor: Pr 55^{gag}
Envelope proteins
  gp 110, p 41
  precursor: Pr 160^{env}
Reverse transcriptase
  p 68
  Mg^{2+} dependent
  preferential template primer: poly A oligo dT_{12-18}
  t-RNA lys. as primer
Viral genome
High-molecular-weight RNA (60s–70s)
2 subunits of 9.2 Kb
6 open reading frames: 5′gag-pol-orfS-env-orfF/3.
Integrated and unintegrated proviral DNA in infected cells

and normal control subjects have been screened for antibodies to LAV/HTLV-III using either enzyme-linked immunosorbent assay (ELISA) (58, 71), Western blot (58, 72), or radioimmunoprecipitation assay (RIPA) using [^{35}S]cysteine-labeled virus (57, 60). Seroepidemiologic studies (Table 2) have clearly established the etiologic association between LAV/HTLV-III and AIDS (58, 71, 73).

Evidence of infection with LAV is well documented serologically in AIDS and ARC patients from Europe, the United States, Zaire, and elsewhere. In contrast, the prevalence of antibodies to LAV/HTLV-III is very low in normal blood donors, and antibodies are not present in patients with non-AIDS-related diseases. The higher rate of seropositivity in African populations (74, 75) has suggested a more important exposure to LAV/HTLV-III infections in this area, and other serologic data have indicated that the virus was present as early as 1973 in Central Africa (75).

In addition, a significant percentage of seropositivity has been found in groups at risk for AIDS, including promiscuous homosexual men, intravenous drug abusers, recipients of multiple transfusions of blood, and hemophiliacs (76, 77). In this last group, the incidence of seropositivity has been clearly correlated with the amount of treatment by antihemophilic factors (76). Although an infinitesimal number of seronegative persons without clinical symptoms were found to be virus-positive in a 1984 study (78), the development of screening of donated blood for antibodies to LAV/HTLV-III has minimized the incidence of the disease in blood and blood products recipients.

Serologic studies have also indicated that, despite repeated exposures to patients with AIDS and specimens, by 1985 no hospital employees or laboratory workers not exposed to needlestick infection were positive for LAV/HTLV-III antibodies (79). This observation is consistent with a low risk of nosocomial transmission of the virus.

The majority of ELISA-positive sera have been confirmed by other techniques, such as Western blot or radioimmunoprecipitation assays. These techniques make it possible to specify which viral antigens are recognized by patient sera. For example, Western blot analysis (Fig. 3) reveals the presence of antibodies to all viral antigens in a patient's sera, but some other sera may contain antibodies only to nonrelated gag proteins such as p 34 or p 41.

The detection of antibodies to gp 110 is easily obtained by radioimmunoprecipitation using [^{35}S]cysteine-labeled virus (Fig. 4). A 1985 preliminary survey of patient sera for antibodies to gp 110 indicated that almost all patients with AIDS had antibodies to this envelope protein (57), even if they were negative for antibodies to the viral core proteins, but positive by ELISA. In a few cases in which sequential sera were available, a disappearance of antibodies to the internal viral proteins was observed at the onset of full-blown AIDS (57). The exact significance of such antibody variations in the prognosis and diagnosis of the disease is still unknown and requires more investigation on a representative number of patient sera.

However, it is clear that the presence of antibodies to LAV/HTLV-III means active viral replication at some time and a risk of transmitting AIDS via blood or sexual contacts.

TABLE 2

*Seroepidemiologic Studies for Antibodies to LAV/HTLV-III Using an ELISA System**

Subjects	No. positive/tested	% positive
Patients with AIDS	83/114	72
Patients with ARC	130/145	90
Sexual partners of AIDS patients	16/34	50
Homosexual men	8/44	18
Hemophiliacs†	85/186	46
IV drug users	5/88	5.7
Zairians with AIDS	32/36	89
Controls		
Normal blood donors	10/11,515	0.1
Zairian controls‡	7/100	7
Patients with infectious mononucleosis	0/44	0
Patients with autoimmune diseases	0/80	0

* Briefly, prediluted sera were tested in duplicate on both viral antigens (purified disrupted LAV virus) and control antigens (a preparation similar to the viral one but from noninfected cells). The specifically fixed IgG were revealed using peroxidase enzymatic reaction. Sera were considered positive when the differential optical densities between the two antigens were higher than 0.3. On each plate, a negative control serum and a positive control serum were tested.

† Both heavily and lightly treated A and B hemophiliacs from France.

‡ Sera from patients treated for noninfectious diseases in Kinshasa in 1983.

Animal Models

Reproduction of the disease in animals exposed to the virus has been considered an important criterion for supporting the implication of LAV/HTLV-III in AIDS.

In 1983 an AIDS-like syndrome (SAIDS) was recognized in some American primate colonies (80, 81), and in 1984 a D-type retrovirus related to, but distinct from, Mazon Pfizer virus was found to be associated with this monkey disease (82, 83).

Although there are numerous differences between human AIDS and SAIDS (83), as well as no antigenic or genome homology between the monkey retrovirus and LAV/HTLV-III, it was of interest to determine whether healthy subhuman primates can develop a disease after inoculation with LAV/HTLV-III. In 1984, infections of chimpanzees with this new human retrovirus and with human AIDS tissues were successful in regard to seroconversion and virus isolation from infected animals (84, 85), but the animals were still healthy in August 1985. The successful infection of a chimpanzee was reported in late 1985 (85a).

Attempts to infect nonhuman primates other than chimpanzees have so far been unsuccessful. However, in 1985 a T-cell tropic retrovirus, referred to as STLV-III, was identified in macaques affected by an immune deficiency syndrome very similar to human AIDS (86). This virus seems to be related to LAV/HTLV-III, and serologic studies have indicated antibodies to this virus in sick animals as well as in a significant number of healthy African green monkeys (87).

The discovery of such a virus in monkeys may provide a model for studies of the basic mechanism of the human viral disease process and for the development of treatment and preventive measures, such as a vaccine.

Prevention and Therapy

Prevention. Further spread of viral infection in some risk groups, such as recipients of blood or blood products, has been minimized by the screening of blood donors. The availability of methods for viral inactivation (88, 89) as well as an increase of hygienic practices in the general population should also prevent possible contaminations by infectious virus.

However, development of a vaccine remains the major requirement for effective prevention. Several laboratories, using different approaches, are actively working on such a vaccine. Although low titers of neutralizing antibodies to the virus have been detected in some patient sera (90, 91), the immunogenic properties of the virus envelope antigen manufactured by recombinant DNA techniques remain to be studied. The genomic diversity among viral isolates is also a determining factor for the development of vaccine.

Therapeutic Approaches. The cytopathic nature of LAV/HTLV-III infection suggests that the best approach to treatment could be to prevent viral spread in noninfected fresh cells.

In this regard, drugs that affect the penetration, the replication, or the assembly of the virus might prove useful. Among such drugs, some inhibitors of viral reverse transcription, such as antimoniotungstate (HPA23), suramin, foscarnet, and ribavirine, are already in clinical trials in AIDS patients (92–94). However, if such treatments are promising for the control of viral replication, they may not be sufficient; procedures to replace lost T lymphocytes (transplantation or immunomodulators) may also be needed. Approaches to prevention and treatment are taken up in detail elsewhere in this issue.

Conclusion

The primary cause of AIDS has been isolated, identified, and characterized. It is a human lentivirus (LAV/HTLV-III) which causes the death and depletion of some T lymphocytes in infected individuals, but, as indicated by the existence of asymptomatic carriers, the primary infection can be inapparent, and only a small population of T cells may be infected and integrate the viral genome.

Thus, many questions remain: how does the virus exert its cytopathic effect, and why, at some point, does the virus rapidly spread in the body, leading to the characteristic loss of T lymphocytes.

Since LAV/HTLV-III is produced only by activated lymphocytes in vitro, it can be assumed that, in a secondary phase (ARC or LAS), viral spread will result from many antigenic stimuli, such as viral or bacterial infections which may stimulate the T-cell system, including LAV/HTLV-III-infected cells. Thus, at this stage, other viral infections, such as those initially thought to be the cause of AIDS (CMV, EBV, HBV) may play an important role as cofactors, although other antigenic stimulation could also be involved (repeated LAV/HTLV-III infections, alloantigens, and so forth). In a third and final phase of the disease, the whole target-cell population, including stem cells, would be infected, and the result of such viral spread would be an irreversible and severe immunodeficiency (AIDS). It is also conceivable that, in these last phases of the disease, cofactors may increase the immune deficiency by autoimmune mechanisms.

However, such a schematic view of the role of LAV/HTLV-III in the pathogenesis of the disease remains to be proved.

AIDS is a complex disease to which many factors, including genetic, immunologic, and environmental, may contribute, but undoubtedly a more complete understanding of the biological and immunologic properties of LAV/HTLV-III and studies on animal models will facilitate control of the disease.

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AIDS: An Autoimmune Pathologic Model For the Destruction of a Subset of Helper T Lymphocytes

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Abstract

Acquired immunodeficiency syndrome (AIDS) is a human retrovirus-induced immune disorder that results in an early loss of T4+ helper T lymphocytes and in the abrogation of immunologic functions associated with a helper T-cell subset. This virus also appears to be cytopathic in vitro, and its primary target appears to be T4+ cells. Based on these findings and recent observations from our laboratory that cytolytic T lymphocyte (CTL) responses to self + X antigens are lost during the early development of AIDS, whereas CTL responses to HLA alloantigens are simultaneously elevated, we develop a model that suggests that the cytopathic effects of the virus and the loss of T4+ cells in the early stages of AIDS are due to the immunopathologic effects of AIDS-virus-specific, HLA self-restricted CTL. The model also proposes an alternate T4+-independent helper pathway that could further promote destruction of virus-infected cells by AIDS-virus-specific CTL without the need for T4+ helper cells.

The evidence is convincing that the primary causative agent for acquired immunodeficiency syndrome (AIDS) is a retrovirus referred to by different laboratories as lymphadenopathy-associated virus (LAV) (1), human T-cell lymphotropic virus-III (HTLV-III) (2–5), AIDS-related virus (ARV) or human immunodeficiency virus (6, 6a–b). Although AIDS is virally induced, the syndrome results in a series of extremely complex immunologic problems that exhibit a spectrum of pathologic manifestations in different individuals and at different times during the course of a patient's disease. For example, patients can have Kaposi's sarcoma (7–9), opportunistic infection (10–12), or both, as well as lymphomas (13, 14) and neurologic disorders (15, 16). Some patients who are seropositive for antibodies to the AIDS virus appear to develop AIDS as defined by the Centers for Disease Control more rapidly than others. Some seropositive individuals have not yet developed AIDS, and it is possible that some will never develop the full manifestations of AIDS. Not only are the consequences of the retrovirus infection immunologic in nature, but it is quite possible that the primary immunologic defect, loss of the T4+ helper-cell component, has an immunologic basis as well (see below).

Since it is probable that infection with AIDS virus results in a primary defect that is immunologic, considerable attention should be focused on various immune-functional aspects of the syndrome, particularly with respect to functional changes that could be attributed to helper T-lymphocyte subpopulations during early stages of AIDS and even earlier (after infection has been detected). Many investigations of helper T-cells in AIDS have been concerned with helper and suppressor T-cell marker analyses (17, 18), with antigen- or mitogen-stimulated proliferative studies (10–12, 18, 19), and with natural killer cell assays (20, 21).

Our efforts in investigating functional abnormalities in AIDS have been focused mainly on

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the in vitro generation of cytolytic T lymphocyte (CTL) responses in homosexual men (22), and more recently in individuals with early signs of AIDS development such as lymphadenopathy and/or antibodies to HTLV-III (Shearer, unpublished observations), referred to here as "prodromal." It could be argued that the generation of cytolytic T lymphocytes is a more complex process than is T-cell proliferation, since the generation of CTL involves more than one T-cell subset. Nevertheless, the CTL approach does offer some advantages, in that antigen specificity can be tested (it is not in the proliferative studies); the suppressor/cytotoxic subset of T cells can be studied for possible defects; and the interaction or absence of interaction between helper/inducer and suppressor/cytotoxic subpopulations can be investigated. Furthermore, it is possible that the cytopathic effects of the AIDS virus have an immunopathologic basis associated with retrovirus-specific CTL (see below). Therefore, any cytolytic T-lymphocyte activity that can be detected in AIDS could be associated with or directed against retrovirus-infected helper T cells (see below). The purpose of this article is to raise some questions and to develop some models of T-cell immunity that may be relevant during the development of AIDS, and to consider some immunopathologic aspects of the syndrome that may be useful to pursue.

Are All T-Cell Responses Defective in "Prodromal" Persons? Since 1982 this laboratory has been involved in a prospective study of CTL response activity in homosexual men. In our model of T-cell immunity, which requires both helper/inducer and suppressor/cytotoxic populations of T lymphocytes, peripheral blood leukocytes (PBL) are sensitized in vitro to influenza virus or to HLA alloantigens by stimulation with a pool of irradiated PBL from multiple donors. Seven days later, the effectors generated are assayed on the appropriate targets in a 6-hr ^{51}Cr-release assay. The CTL response to influenza requires recognition of viral antigens in association with self HLA antigens (23) and is therefore referred to as a "self + X" response, whereas the response to HLA alloantigens appears not to be self-restricted. Since we have used PBL from a pool of eight HLA-disparate donors for allogeneic stimulation, the allogeneic CTL response is referred to here as "allopool."

We reported in 1984 that approximately 25% of homosexual men who do not have AIDS or symptoms of AIDS exhibited reduced CTL responses to self + X, compared with no reduction in CTL activity in heterosexual men (22). More recently, we have observed that this reduction in self + X CTL activity was not correlated with donors who were seropositive for antibodies to HTLV-III (23a).

However, we also observed that the only two donors in that study who exhibited a total absence of self + X CTL activity were both seropositive for HTLV-III antibodies; both had T4:T8 ratios of 0.4, and both had a 3-year history of lymphadenopathy. The T4:T8 reversal was due to a loss of T4+ cells. Furthermore, retrovirus activity was detected in PBL cultures from both donors (23a).

The most surprising aspect of the functional immune profiles of these two donors was that both exhibited elevated CTL activity to HLA alloantigens. Note that the CTL response to multiple alloantigens by PBL from heterosexual donors is relatively weak and seldom exceeds 20 lytic units/10^7 cells. This contrasts with the CTL responses to allopool of homosexual men, more than 50% of whom exhibit elevated CTL activity to allopool (as well as to alloantigens from single donors) in the range of 80–300 lytic units/10^7 cells (24). Note also that proliferative responses to the allopool were not elevated in the PBL that exhibited elevated CTL activity, but PBL from these donors maintained more potent proliferative responses for an extended period of the kinetic curve than did PBL from normal CTL responders (Shearer et al, unpublished observations). Furthermore, in more recent studies we have observed that PBL from all patients with AIDS thus far tested are unable to generate CTL responses to self + X, whereas PBL from approximately half of the same AIDS patients generate elevated CTL responses to allopool (Shearer, unpublished observations).

Based on these findings, it appears that not all AIDS patients will exhibit T-lymphocyte immune defects to all antigens. It is possible that during the development of AIDS the first CTL responses to be lost will be those that involve recognition of foreign antigens (including pathogens) that are recognized in association with self major-histocompatibility-complex (MHC) determinants, and that only in the later stages of the syndrome will HLA allogeneic CTL responses be reduced. Thus, there appears to be a selective loss of self + X CTL responses during the development of AIDS.

What Mechanism Is Responsible for "Prodromal" Selective Loss of Self + X CTL? The selective loss of self + X CTL with an elevation in allopool activity could be due to one of at least

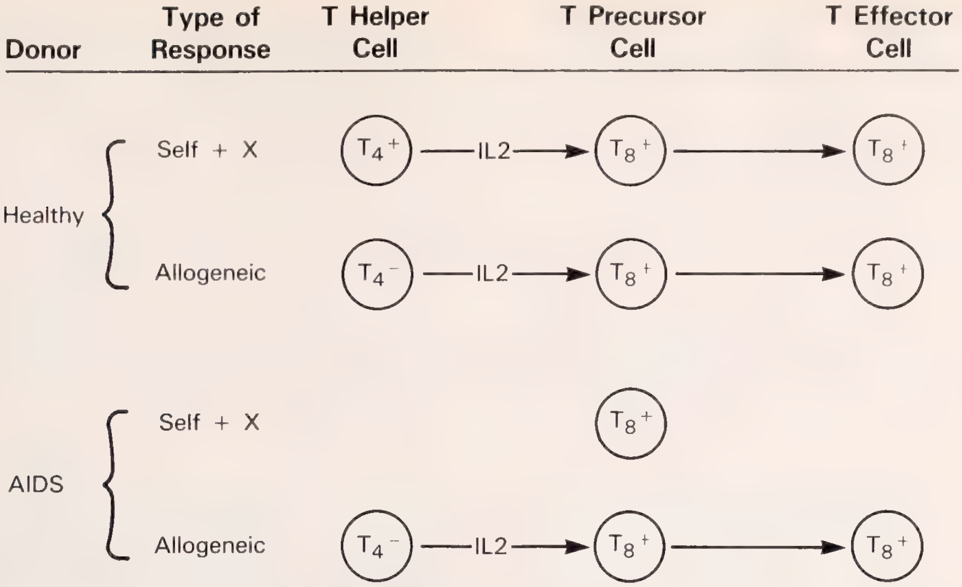


Fig. 1. Possible T4+-dependent and T4+-independent helper T-cell pathways in healthy persons and absence of T4+ helper subpopulation required for self + X CTL responses in AIDS patients.

two mechanisms. First, the fact that we have observed a high proportion of homosexual men with elevated allopool CTL activity who do not have AIDS raises the possibility that these donors have been primed to HLA alloantigens as a result of their sexual practices (24, 25). It is possible that primed CTL responses are less dependent on T4+ helper cells and that the AIDS-associated reduction in T4+ cells will not have an initial detrimental effect on allo-CTL function. A second possibility, which is not necessarily mutually exclusive with the first, is that CTL responses to self + X and HLA alloantigens can utilize different helper T-cell pathways, and that a T4− helper cell pathway can be utilized for CTL responses to HLA alloantigens.

Singer and his co-workers have developed an alternate-pathway model for the generation of CTL to self + X and H-2 alloantigens in the mouse (26, 27) that may be relevant for the selective loss of self + X CTL responses during AIDS development. In this model, CTL responses to antigens that are recognized in association with self H-2 determinants (self + X) necessarily utilize L3T4+ helper T cells (considered the murine equivalent of human T4+ cells). However, CTL responses to H-2 alloantigens can utilize either L3T4+ helper T cells, or, alternatively, they can utilize Lyt2+ helper T cells (considered the murine equivalent of T8+ cells) (26, 27). Thus, by depleting murine T cells of the L3T4+ subpopulation, these investigators were able to abrogate CTL responses to self + X antigens while maintaining strong CTL activity to alloantigens (26, 27).

Similarly, recent studies indicate that immune deficiency induced in mice by a chronic graft-versus-host reaction that involves only recognition of class II major histocompatibility antigens results in the selective loss of self + X CTL responses (28). Allogeneic responses remain at normal levels or are elevated. In both these murine models, self + X CTL activity can be restored in vitro by the addition of interleukin-2 (IL-2)-containing supernatants, suggesting that only helper T cells are affected. These observations are similar to the in vitro effects of IL-2 on generation of CTL in AIDS patients (20).

The parallels observed in our donors who may be developing AIDS and in these murine models raise the possibility that a T4-independent helper-cell pathway may exist in humans for the generation of allogeneic T-cell responses (Fig. 1). Infection with the AIDS virus would deplete the patient of T4+ helper T cells and would abrogate any immune responses that require this helper-cell population. If an alternate T4-independent helper-cell pathway exists for certain antigens, immune responses that can utilize this pathway should be demonstrable in AIDS patients. It is probable that later stages of the syndrome will also deplete other T-cell populations and destroy all immune function. In fact, a T4-independent alternate helper pathway appears to

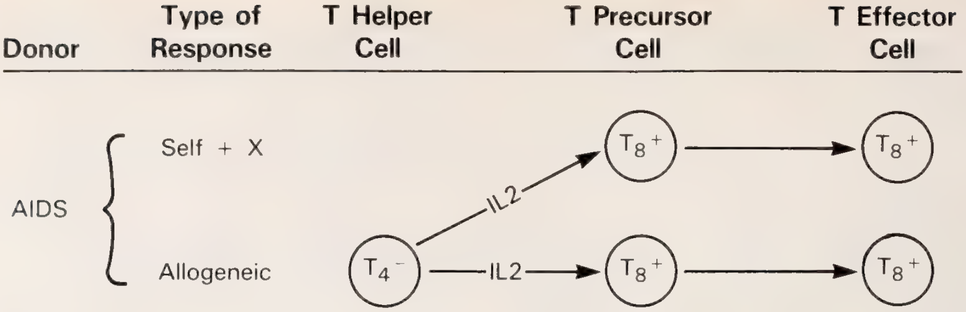


Fig. 2. Above Model illustrating possible activation of self + X CTL effector pathway in AIDS patients who lack T4+ helper T cells. Interleukin-2 (IL-2) is activated by allostimulation of T4− allogeneic helper T cells. Effector T cells specific for self + X antigens are activated by self + X antigens.

have been found for the CTL response to influenza virus (29).

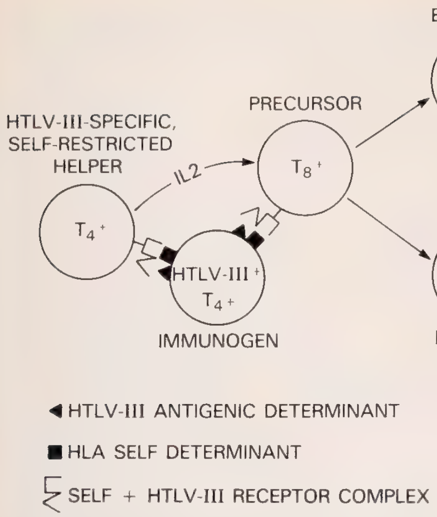
Could the Elevated "Prodromal" Allogeneic T-Cell Responses Be Used to Enhance Immunity to Infectious Agents? If an alternate helper T-cell pathway such as the one postulated above exists for T-cell responses to HLA alloantigens, it is possible that this intact helper-cell pathway could be utilized to provide help for immune responses to antigens that normally require T4+ helper cells. This approach takes advantage of the demonstration in murine models of helper and cytotoxic cell interactions such that helper T cells specific for one antigen can be used to provide help for the generation of CTL to a second antigen if both antigens are administered simultaneously (30, 31). Thus, if helper cells specific for one antigen are absent but effector cells to this antigen are intact, and if both helper and effector cells specific for a second antigen are intact, it is possible to generate help through IL-2 production for both populations of specific effectors. This can be achieved by simultaneous activation of helper T cells and effectors specific for the second antigen and effectors specific for the first antigen (Fig. 2).

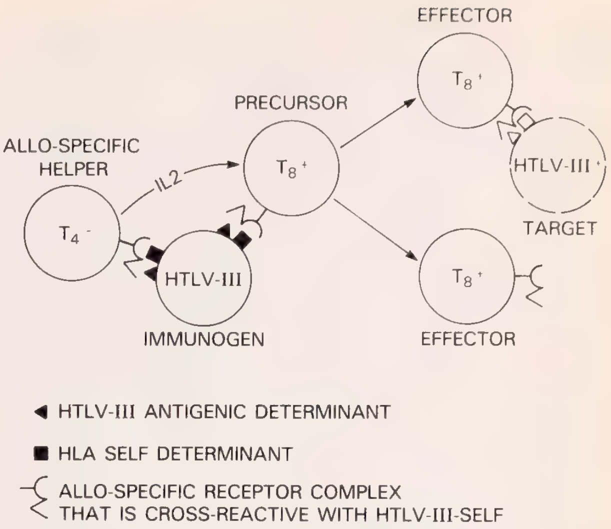
Since some homosexual AIDS patients and several homosexual men with lymphadenopathy exhibit elevated CTL to HLA alloantigens, the possibility exists for enhancing immune responses to pathogens by coimmunization with pathogen-specific antigens and HLA alloantigens in those patients who demonstrate elevated CTL or prolonged proliferative responses to HLA alloantigens. Immunization with HLA alloantigens might be successfully administered in the form of blood transfusions.

Is the Cytopathic Effect of the AIDS Virus on T4+ Cells due to Cytopathic Properties of the Virus or to Immune-Induced Destruction of Virus-Infected T Cells? It has been demonstrated that infection of PBL in vitro with HTLV-III results in an initial increase in virus production, followed by a reduction in virus production associated with death of the cells in the cultures (2, 32, 33). Furthermore, it has been observed that persons with AIDS lose T4+ cells and become leukopenic (17, 18). These findings have been interpreted to indicate that the AIDS virus is cytopathic, although the mechanism by which it is so is unknown.

An immunologic mechanism that could account for the apparent cytopathic properties of the AIDS virus is the development, in the early stages of the syndrome, of CTL that would be specific for antigenic determinants of the AIDS virus recognized in association with self HLA determinants (that is, virus-specific, HLA-restricted cytotoxic effectors). This possibility is based on the CTL-induced immunopathologic murine model of lymphocytic choriomeningitis virus (LCMV) discovered and developed by Doherty and Zinkernagel (34). In this model, the immunopathogenic effect of the virus is mediated by CTL that are specific for the virus and restricted to self MHC antigens. It is noteworthy that athymic nude mice deficient in helper T cells do not develop the immunopathologic effects of LCMV (34).

A similar model can be developed for AIDS in which the patient's own virus-infected T4+ lymphocytes, which appear to be the primary targets of the AIDS virus infection, would also become the targets of virus-specific, self HLA-restricted cytolytic effector cells (Fig. 3). Thus, the cytopathic effect of the AIDS virus which is most pronounced on infected T4+ cells in tissue culture (33) and early in the syndrome may not be exclusively due to a cytopathic effect of the virus per se and may also include immunopathologic effects of T-effector-cell destruction of the cells primarily infected by the virus. If the virus is cytopathic in vitro on a population of T4+-


EFFECTOR
PRECURSOR
HTLV-III-SPECIFIC, SELF-RESTRICTED HELPER
IL2
T4+
T8+
HTLV-III+ T4+
TARGET
HTLV-III+ T4+
IMMUNOGEN
EFFECTOR
◀ HTLV-III ANTIGENIC DETERMINANT
■ HLA SELF DETERMINANT
SELF + HTLV-III RECEPTOR COMPLEX


EFFECTOR
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HTLV-III+
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IMMUNOGEN
EFFECTOR
◀ HTLV-III ANTIGENIC DETERMINANT
■ HLA SELF DETERMINANT
ALLO-SPECIFIC RECEPTOR COMPLEX THAT IS CROSS-REACTIVE WITH HTLV-III-SELF

Fig. 3. Left Model illustrating HTLV-III-specific, HLA self-restricted pathway for generation of CTL that could destroy autologous T4+ T cells infected with HTLV-III.

Fig. 4. Right Model illustrating allospecific T4− cells that could cross-react with HTLV-III-infected autologous cells. Such cross-reactions could occur at T helper or CTL precursor level, or at both (as shown).

enriched, T8+-depleted cells, the cytopathic effect is not likely to be due to an immunocytopathic mechanism. However, even with such a demonstration, comparing in vitro models with in vivo mechanisms might not be valid.

It could also be argued that such a model should be self-limiting, since the generation of these putative CTL would require helper T cells of the T4 phenotype, and this helper T-cell function would be of limited duration owing to destruction of T4+ helper cells. However, in the initial stages of AIDS virus-specific CTL activation, helper T-cell function should be intact and could augment the generation of the AIDS-virus-specific CTL. Even during later stages of the syndrome, when the number of T4+ cells is decreasing, the presence of any T-cell immune response that would elicit the production of relevant lymphokines, including IL-2, could augment CTL activity of the putative AIDS-virus-specific effectors, particularly if the following were present: immunogen (AIDS virus + self HLA), effector T cells specific for AIDS virus + self HLA, and immunogen and activated helper T cells for another unrelated T-cell response.

Thus, it is possible that any T-cell immune activity that remains intact or is enhanced during the development of AIDS (such as the elevated allogeneic CTL responses noted above) could provide helper signals that would promote further CTL-mediated destruction of autologous lymphocytes that express AIDS viral antigenic determinants. It is also possible that an infected individual could remain in a relatively stable condition until the appropriate antigenic stimulus activated any remaining helper T cells that would, in turn, activate AIDS-virus-specific CTL and destroy the patients' T cells. Furthermore, if antigenic cross-reactivity were to occur (particularly at the helper-cell level) between antigens for which T-cell immunity remains intact or is enhanced in AIDS patients and the AIDS virus (a possible candidate is HLA alloantigens), one would have the potential for continuous stimulation and help for AIDS-virus-specific CTL.

Could Protocols that Circumvent the Need for or that Enhance Helper T-Cell Function in AIDS Be Detrimental to Patients' Immune Status? Attempts are being made in patients with AIDS to enhance T-cell-mediated immune function by administration of recombinant IL-2 (35). An alternative approach to repeated inoculation of IL-2 (which can have undesirable side effects) is possible in those patients who exhibit elevated T-cell immunity to HLA alloantigens (Fig. 2). This approach could involve attempts to immunize with HLA alloantigens, possibly by blood transfusions. However, it should be noted, as discussed above, that protocols that enhance helper T-cell function have the potential for augmenting putative cytolytic T-cell activity against AIDS-virus-infected autologous T4+ lymphocytes. Thus, attempts to enhance helper T-cell function could have an overall detrimental effect on the patient's immune system by enhancing cytolytic T-cell activity against virus-infected T4+ cells.

Would an In Vitro CTL Model Be of Value for Studying AIDS? If the cytopathic effects of the virus are the result of immunopathologic consequences of CTL rather than of direct cytopathic effects of the virus, and if attempts to augment helper T-cell function have the potential for enhanced killing by these putative CTL, efforts should be made to develop an in vitro model for demonstrating and testing for the presence of such CTL. Retrovirus-specific CTL responses have been demonstrated in the blood of patients in remission with adult T-cell leukemia (36). These CTL were HTLV-I-specific and HLA self-restricted. Thus, a CTL model has been developed for a retrovirus of the HTLV series, and it may be possible to develop a similar model for HTLV-III/LAV. Once developed, this test should be useful for monitoring the presence of CTL in various stages of AIDS development. It could be determined whether CTL activity was correlated with a loss of T4+ cells in the patient, and whether protocols that enhance helper T-cell activity also enhance virus-specific CTL activity. Finally, such a CTL model could be used to test whether the cytopathic effects observed in vitro on infected T cells are due to the virus itself or to cytotoxic effector cells.

Could Persons Primed to HLA Alloantigens Be Particularly Susceptible to AIDS? It is possible that persons who exhibit elevated CTL activity to HLA alloantigens are at greater risk for developing AIDS than those who exhibit lower allogeneic T-cell responses. Increased susceptibility to AIDS in this group of individuals could be visualized by one of at least two mechanisms.

First, since the in vitro infection with HTLV-III/LAV can be achieved more efficiently in mitogen-stimulated than in unstimulated cultures, it is possible that T lymphocytes in alloprimed individuals will be more efficiently infected with HTLV-III/LAV in vivo than T cells in unprimed individuals. Furthermore, PBL can be more efficiently infected in vitro with HTLV-III/LAV after stimulation with a pool of HLA alloantigens than after stimulation with mitogen (Shearer et al, unpublished observations). Thus, the possibility exists that alloprimed donors may be more susceptible to HTLV-III/LAV infection than unprimed donors.

Second, it is possible that the CTL to HLA alloantigens are cross-reactive with AIDS-virus-infected autologous cells. However, it has been shown that homosexual men can exhibit elevated CTL activity to alloantigens without being seropositive for antibodies to HTLV-III/LAV (24). Thus, priming to HLA alloantigens is not necessarily the result of exposure to the AIDS virus. This, however, does not exclude the possibility that allogeneic CTL effectors would cross-react with and lyse HTLV-III-infected self targets. As noted above, some AIDS patients exhibit elevated CTL activity to alloantigens, although they are deficient in responses to self + X antigens (Shearer, unpublished observations). If cross-reactions exist at the effector-cell level between HLA alloantigens and HTLV-III/LAV-self, alloprimed CTL could kill infected T4+ cells and contribute to the loss of T4+ lymphocytes in AIDS patients in much the same way as the putative HTLV-III/LAV-specific, HLA-restricted CTL discussed above. Cross-reactions could also occur between alloantigens and HTLV-III/LAV-self at the helper T-cell level. If this is true, then the immune system might not require functionally intact T4+ cells to provide help for HTLV-III/LAV-specific effector cells, since putative allo-specific T4− helpers could provide such help (Fig. 4). It is also possible (although not yet demonstrated in the murine model) that a self + X CTL response could be initiated via a T4− helper-cell subset (29), which would function without the need for T4+ helpers.

Conclusions

This discussion has considered certain factual and hypothetical aspects of T-cell immunity that may be relevant to the development and the treatment of AIDS. Many of the points covered here have not been pursued by those working with AIDS in clinic or laboratory, yet understanding of the questions raised could be important if the early stages of AIDS are to be successfully treated. It is hoped that some of the issues raised will provide worthwhile avenues of investigation.

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Neurologic Effects of HTLV-III Infection in Adults: An Overview

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Neurologic complications of AIDS have been recognized since the first reports by Britton et al and Horowitz et al (1, 2) in 1982 and Herman (3) and Bredesen and Messing (4) in 1983. Characteristic clinical syndromes affecting the brain and spinal cord and nonspecific peripheral nerves and muscle signs and symptoms originally described by Snider et al (5) were recently reviewed by Levy et al (6).

The early neurologic manifestations of the central and peripheral nervous system are often not identified; neither their time course nor their prognostic implications have been sufficiently documented. Because the search for effective therapeutic modalities and for a vaccine have so far been unsuccessful, and because it is not uncommon for the first manifestations of AIDS to involve the nervous system (7), recognition of early nervous system involvement is increasingly important. Molecular hybridization techniques have documented more extensive involvement by HTLV-III in the brain than in other target organs such as the lymph nodes, lung, or liver (8).

In a recent national survey of the neuroepidemiology of AIDS in 16,574 adult cases as of January 1986 (9), 10% of all patients initially had neurologic complaints, 40% developed major neurologic symptoms, and 80% had nervous system pathology at autopsy. Central nervous system complications were as follows: cryptococcosis 4.3%, toxoplasmosis 2.25%, progressive multifocal leukoencephalopathy 0.50%, and primary central nervous system lymphoma 0.50%. The most common complications today, dementia (originally called "subacute encephalitis") and myelopathy, were not addressed.

This study and others (7–9) have raised the question of a neurotropic variant for this slow retrovirus, whose clinical effects have been likened to visna, a lentivirus responsible for a slow, degenerative nervous system disease of sheep (10, 11).

The median survival time in a New York City survey (12) of 3,204 unselected AIDS patients was 261 days from the date of diagnosis; the probability of death decreased after three years, and—for the few survivors—leveled off after four years. Few patients with central or peripheral nervous system involvement are alive after two years (7–9). Such survivors are of special interest, since they may shed light on natural therapeutic processes. One such possibly qualifying case with myelitis is detailed below.

This overview summarizes some of the early important papers in the AIDS literature and some of the presentations at the International Conference on AIDS in Paris, June 1986.

The following classification is offered for ease of description only, since most neurologic syndromes that occur in pre-AIDS—that is, AIDS-related complex, or generalized lymphadenopathy, or benign lymphadenopathy—will eventually be found in full-blown AIDS.

- Neurologic syndrome in seroconvertors.
- Neurologic syndromes in pre-AIDS.
- Primary neurologic syndrome in pre-AIDS or AIDS: dementia, myelopathy, dementia myelopathy complex, CNS lymphoma, myopathies, Guillian-Barré-like syndrome, myelitis.
- Neurologic syndrome complicating full-blown AIDS: meningitides, cerebral complications of systemic viral illnesses.
- Cerebral vascular lesions: progressive multifocal leukoencephalopathy, other syndromes.

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Neurologic Syndrome in Seroconvertors

There is little information on this subgroup, but recently Cooper et al (13) have defined the clinical illnesses associated with seroconversion in a group of 38 homosexuals from Australia. Lethargy (90%), malaise (83%), fever (70%), sore throat (67%), and myalgia (66%) were most common, followed by anorexia (60%), arthralgia (50%), and headaches (53%). No follow-up information or details of the neurologic syndrome were available.

Ho and his group (10) have recovered HTLV-III from the cerebrospinal fluid of seroconvertors during acute aseptic meningitis. One of the patients who made a full recovery was free of opportunistic infections, but developed generalized lymphadenopathy.

It seems reasonable that previously healthy homosexuals with a prolonged "flu-like" syndrome should have a full neurologic examination. Spinal tap should be considered to rule out invasion of the central nervous system by LAV or opportunistic infections. This is, moreover, a most important group to diagnose, since at this stage therapy, when available, may be most effective.

Neurologic Syndromes in Pre-AIDS

Pre-AIDS, in my reading of the consensus, is being defined as a person who tests positive for antibodies to HTLV-III and has generalized lymphadenopathy and may also have a pure neurologic syndrome, and is immunodepressed.

The rate of conversion from pre-AIDS to AIDS has been estimated at between 2% and 10% each year for consecutive years, with no decrease over the years (14, 15).

In a San Francisco study of 372 patients (14) with AIDS-related complex (ARC), 17% developed AIDS at a mean of 21 months of lymphadenopathy. The peak rate of progression to AIDS occurs at about one year after the onset of lymphadenopathy at a rate of 2.4% per year. In a New York study (15) in which 257 homosexuals have been followed up since 1981, 12% had AIDS by 1983 and 23% by December 1985, for an approximate rate of 10% per successive year.

The neurologic signs and symptoms in 15 patients with ARC (16) included 7 with memory loss, 2 with herpes zoster, and 1 with peripheral neuropathy. Several had cognitive difficulties; mental testing results were normal in only about half these patients.

Polyneuropathy manifested by paresthesis and weakness in the limbs is reported in a Paris multihospital study of five patients (17). In all five, distal weakness with arreflexia and sensory abnormalities were associated with pleyocytosis, high protein, and normal sugar in the cerebrospinal fluid. EMG showed reduced velocity, and sural nerve biopsy showed segmental demyelination with perivascular infiltrates. Symptoms remained stable in four patients. One patient died of AIDS 20 months after the onset of neuropathy. But one patient's polyneuropathic symptoms resolved on oral prednisone, an astounding outcome—although another French group (18) has also noted regression of lymphadenopathy in 11 patients with ARC in a group of 128 patients.

The headache syndrome has not been fully characterized in ARC (19) and has often been labeled "tension headache" or headache secondary to associated depression and anxiety (20).

A CT scan of the head is not commonly performed in pre-AIDS patients. Similarly, no data on EEG is available, although EEG may provide earlier clues to the slow virus encephalopathy of pre-AIDS.

A 42-year-old homosexual was seen by the author in July 1986 for evaluation of changes in personality and depression. He was in good physical health and working. His physical examination and laboratory results were normal, save for a 1/1000 titer for the Epstein-Barr virus. Neurologic examination was remarkable for mild dysarthria and slowing and hesitancy in response to simple questions. There were no motor signs of impairment. An EEG showed bilateral 4–5 cps slowing. He refused an ELISA. In September 1986, he developed herpes zoster radiculitis but continued to work.

Transient unilateral or bilateral facial paralysis, either isolated or accompanying aseptic meningitis, has been described in ARC; the author has seen such paralysis in two patients. Spontaneous resolution in one and with prednisone in the second followed within one month. All the patients were reported by Ho et al (10) to have done well. The reason for the frequent involvement of the facial nerve in pre-AIDS is not clear. Bell's palsy in high-risk individuals warrants an ELISA and a spinal tap if meningeal signs are present.

Raeder's syndrome has been described (21) in two patients, both of whom developed AIDS within one year and died within two years of onset, one with pneumocystic pneumonia and a cerebral tuberculoma, the other secondary to cryptosporoidosis.

Myelopathy and Myelitis. Ascending myelitis resulting in quadriplegia was reported in a patient with Kaposi's sarcoma (22). Cerebrospinal fluid cultures yielded cytomegalovirus, confirmed by postmortem cultures from brain and spinal cord which also grew herpes simplex virus II.

Levy et al (9) reported a case of a patient with transverse myelitis who is alive and well. No detailed clinical description is given.

Two cases of myelitis secondary to epidural metastatic lymphoma were reported by Snider et al in 1983; both died (5).

Case Report: Sustained Recovery from AIDS? In 1983, I reported on a 45-year-old homosexual with thoracic myelopathy secondary to an epidural mass (3). At surgery in December 1981, a mass wrapped around the cord was excised. The pathologic finding was chronic lymphocytic and histiocytic infiltration (11). "Chronic inflammation likely but a neoplasm such as lymphoma cannot be entirely excluded," read the pathology report. An immune deficiency consistent with AIDS was diagnosed in 1982 when he was rehospitalized with liver cirrhosis. He was treated with steroids and a Denver shunt and made a remarkable sustained neurologic recovery. His helper-to-suppressor T-cell ratio at present is normal and his ELISA as of 1985 was negative.

This patient was known to carry hepatitis B virus. His case raises the important question whether competitive inhibition of hepatitis B virus and HTLV-III for the same T cell or the large amounts of antigen surface produced by hepatitis B virus and attached to the outer coat can block the penetration of HTLV-III into the T cell and avert its destruction.

Indeed, it is remarkable that in spite of almost universal hepatitis B markers in male homosexuals and AIDS patients reported by a French group (23), very few cases of overt hepatitis among either population are on record. The same group of French workers (23) report the presence of hepatitis B virus in T cells. Experimental studies of co-culture of these two viruses should be enlightening and are now in progress at Mount Sinai in the laboratories of Dr. S. Hirshman; other researchers consider hepatitis B virus a co-factor in the development of AIDS (24).

Primary Neurologic Syndrome

Dementia. Dementia (originally, "subacute encephalitis") was described by Snider et al (5) at Sloan-Kettering. Further work in New York by Navia and Price (25) and Petito et al (26) analyzed in great detail the AIDS dementia complex and the vacuolar myelopathy syndrome. Today these two syndromes, often combined, are the most common neurologic complications of AIDS (25, 26). Either the dementia or the myelopathy may be the initial symptom of AIDS, but the exact time relationship of either symptom and immunodeficiency is not yet established.

The dementia has an insidious onset and runs a slow protracted course of months, rarely extending to two years. Initial manifestations include behavior changes, loss of memory, apathy, and depression. In 50% of the patients, motor-slowing clumsiness of limbs and ataxia are present. Lethargy with frank psychomotor retardation ensue. Rarely are the systemic diseases sufficient to account for the neurologic picture. Dementia with mutism, seizures, paraplegia with incontinence, and recurrent infections with malnutrition lead to death (25, 26).

At autopsy, palor and vacuolation, along with astrocytosis and demyelination of white matter and gliosis of the cerebral cortex and subcortical nuclei, are the prevalent findings (27). LAV RNA has been detected in large cells distinct from T4 cells in the brain of AIDS patients (28).

In 24 of the 70 patients described by Navia and Price (25), neurologic impairment was not clinically documented. In milder cases of dementia, perivascular inflammatory changes were present; in severe cases, multinucleated cells were prominent.

Computerized tomography and magnetic resonance imaging reveal cerebral atrophy (29). Recently, positron emission tomography (30) has documented decrease in brain glucose metabolism paralleling the clinical dysfunction and affecting mostly the frontal and temporal lobes.

Myelopathies. The vacuolar myelopathy resembling subacute combined degeneration (26, 31) is identified today in a third of AIDS patients with dementia and has been found in 22% of consecutive AIDS autopsies (25). It is a primary affliction of the thoracic pyramidal tract and posterior columns resulting in progressive ataxia and flaccid paraparesis. Associated proprioception difficulties and hyporeflexia or hyperreflexia eventually bind the patient to a wheelchair. Dementia develops in up to one half. Outcome is fatal, usually within one year. A full-blown immune deficiency complicated by opportunistic infection is always found, in some patients after onset of myelopathy. At autopsy, typical vacuoles in the white matter are surrounded by thin myelin sheaths. HTLV-III has been cultured from the spinal fluid and the spinal cord at autopsy (26).

CNS Lymphoma. Central nervous system lymphoma was first described by Horowitz (1) and Britton (2) and then by the Sloan-Kettering group (5). These tumors are seen with increasing frequency; a California study (32) reported that among 27 homosexuals with lymphoma, 87% were HTLV-III positive. Of the 27, 8 had original central nervous system involvement and 4 had extension into the nervous system for a total of 44%. The average survival was 8 months only, in spite of chemotherapy and radiation. Only one of these 27 patients was in remission 28 months later.

Polyneuropathy. A Guillian-Barré-like polyneuropathy in AIDS was described in 1982 and 1983 (1–3). My first AIDS patient, a 44-year-old black homosexual, was hospitalized in March 1981 with distal weakness, arreflexia, and hypoesthesia in the legs. A spinal tap showed protein of 200 mg/ml, normal sugar, and 19 white blood cells (92% lymphocytes); he incidentally tested positive for syphilis in serum (but negative in cerebrospinal fluid). He was treated with intravenous penicillin (10 million U) daily for 10 days. He was subsequently readmitted, for "recurrent Guillian-Barré," from which he recovered on oral prednisone, and then for fever of unknown origin, generalized lymphadenopathy, and immunologic evidence of AIDS. A left parotid gland swelling that was biopsied showed virus-like particles on electron microscope. He died with pneumonia, urinary tract infection, and dementia in 1983.

In 3 patients reported by Eidelberg et al (33), the duration of illness was shorter, ranging from 4 to 10 months. In one, cytomegalovirus was identified within the Schwann cells of a lumbar root.

Recently, plasmapheresis has been reported (34) to produce good results in four of six patients with polyneuropathy and ARC or AIDS.

Myopathies. Progressive proximal weakness in facial, neck, and limb muscles of three patients with AIDS-related complex or AIDS was reported by Simpson et al (35). The CPK was elevated and EMG revealed denervation and myopathy. Biopsy in one patient revealed inflammation. Two patients responded to prednisone alone, one patient to prednisone plus azathioprine. A second group of three homosexual patients with proximal myopathy and positive ELISA had EMG evidence of denervation and myopathy, confirmed by muscle biopsy in one. One is doing very well on a maintenance dose of 20 mg prednisone, having regained strength in shoulder and thighs, though muscle atrophy persists.

Neurologic Syndromes Complicating Systemic AIDS

Nonviral central nervous system lesions complicating AIDS were the first to be recognized (1–5) and have been abundantly described. Today, they are second in frequency to AIDS dementia and myelopathy.

Transient aseptic meningitis was described by Snider et al (5) and others (6, 10, 13). Long-term outcome is not known. In two patients, bilateral transient VII nerve paralysis was present, along with headaches, fever, and stiff neck (10). Cerebrospinal fluid is always abnormal, monocellular pleyocytosis ranging up to 300 cells/mm^3 and protein being in the 100 mg/ml range; glucose is normal or low. All patients in this series recovered within one month, although further follow-up is not reported. One of these patients had had four bouts of meningitis.

Cryptococcal meningitis is the most common fungal infection in AIDS. Progressive disabling headaches, at times extraordinarily painful, are common and respond best to repeated spinal taps. This relentless disease is characterized by fever, headaches, poor clinical response with frequent relapse and a usually fatal outcome. Neurologic features include meningismus, visual and mental impairment, and cranial nerve palsies; one case of papillodema was seen in the 33 patients of the San Francisco group (36). Cerebrospinal fluid pressure was usually slightly elevated, with protein averaging 60 mg/ml, glucose 50 mg/ml, and monocellular pleyocitosis about 20 cells/mm^3. The India ink preparation, a must in all high-risk patients, was positive in 24 of the 25 patients who were so tested.

Serum antibody titer is only a fair index of therapeutic response. Intravenous ketoconazole or amphotericin is the treatment of choice (37). In only two cases of 33 reported did the fungus clear completely from cerebrospinal fluid (36). Fungemia without meningitis was also described in this series. CT only rarely reveals intracerebral masses; none were seen in the Levy series (29). Hydrocephalus is seen occasionally, as well as atrophy. One patient now in The Mount Sinai Hospital has developed a documented cerebral small lesion. *Mycobacterium avium* with brain involvement has been described as a rarity (3, 5, 6). This novel disease frequently affects the lung and liver in AIDS. Triple antitubercular therapy has been used in these patients, who nevertheless succumb to opportunistic viral infections.

Recently, a significant increase in the incidence of pulmonary tuberculosis secondary to *Mycobacterium tuberculosis* in the AIDS population has been recognized (38), with occasional central nervous system involvement and tubercular meningitis. In spite of high doses of conventional therapy, reported survival is short.

Cerebrovascular Lesions

Cerebrovascular complications in AIDS are rare; hemorrhagic or ischemic infarcts were reported in patients with thrombotic endocarditis (6), lymphoma (5), and Kaposi's sarcoma (9). The patient with thrombotic endocarditis responded to anticoagulation treatment.

Vascular insults are also seen in drug addicts with AIDS and bacterial endocarditis, or in association with encephalitis due to cytomegalic virus or lymphoma (5, 9).

A few cases of progressive multifocal leukoencephalopathies with documented myxoma virus on brain biopsy are on record (5, 6, 39); treatment has been universally unsuccessful.

Other viral infections that occasionally extend to the central nervous system include herpes simplex, type I, producing meningoencephalitis, and cytomegalovirus encephalitis (1, 4–6). Bacterial involvement of the central nervous system is surprisingly low in these immunodeficient patients (5, 6). Occasional bacterial abscesses have been reported, but they are infrequent. Rare instances of Kaposi's sarcoma with intracranial metastasis, which are rapidly fatal, are reported (6).

Various syndromes, including ataxic gait, tremor, Parkinson-like syndrome, and seizures, have been described in AIDS (1, 2, 5, 6). None are sufficiently specific to merit a separate designation, nor do they point to any particular etiologic co-factor.

Toxoplasmosis was among the early recognized central nervous system complications of AIDS (1, 4–6, 40). Multiple ring-enhancing lesions in the brain and cerebellum are present. Clindamycin therapy with sulfadiazine or pyramethamine is successful in reducing the size of the lesions or actually completely eradicating them. Nevertheless, all these patients succumb.

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Is Cytomegalovirus a Cofactor in the Pathogenesis of AIDS and Kaposi's Sarcoma?

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In the first part of this review, I summarize the evidence suggesting a possible cofactor role for cytomegalovirus (CMV) in acquired immune deficiency; in the second part, the role of this virus in the pathogenesis of Kaposi's sarcoma.

Is CMV a Cofactor in AIDS?

The evidence linking CMV to AIDS is as follows.

High Prevalence of CMV Infection Among Homosexual Men and Patients with AIDS. The initial documentation of high rates of CMV infection in homosexual men was in prevalence studies performed by my group at the San Francisco Venereal Disease Clinic in 1979 (1). We observed urinary excretion of CMV in 14 of 190 (7.4%) homosexual men, but in none of 101 heterosexual men attending the same clinic ($p < 0.005$). Similarly, antibody to CMV was detected in 130 of 139 (93.5%) homosexual men, but in only 38 of 70 (54.3%) heterosexual men ($p < 0.005$).

In a subsequent prospective study (2), 22 of 31 homosexual men with serum negative for CMV had conversion to positive serum within 9 months, an attack rate of 71% during this period. In a mean follow-up period of 14.3 months (range, 2–20), 66 of 206 initially seropositive men (32%) excreted CMV in their urine on one or more occasion. Both a urine and a semen specimen were obtained during a single visit from 62 of the homosexual men. CMV was recovered from 14 of the semen specimens, but from only 2 of the 14 corresponding urine samples. Semen therefore appears to be approximately five times as sensitive as urine for detecting the presence of CMV. Clearly, the widespread occurrence of CMV viruria and "virusemenia" in this population makes exposure to the virus all but inevitable and accounts for the extraordinarily high attack rate of CMV infections among seronegative homosexual men.

Of 164 AIDS patients (including homosexual men, intravenous drug users, and hemophiliacs) tested by the Centers for Disease Control for CMV antibody, 162 were positive (3). The 2 with negative results may have been end-stage AIDS patients who, in our experience, may be unable to maintain antibody responses.

Occurrence of Reinfection (New Primary Infection) with Different Strain of CMV. Given the high rates of virus in semen cited above, homosexual men may be repeatedly exposed to, and possibly infected by, differing strains of CMV. Our data on the prevalence of CMV IgM antibody suggest that homosexual men experience repeated episodes of CMV infections; CMV IgM antibody was detected on one or more occasion in the sera of more than 90% of the homosexual men and tended to appear, disappear, and then reappear again over the course of time (2). In contrast, IgM antibody to CMV was detected in only 3.8% of 103 serum specimens randomly collected from volunteer male blood donors. Among the ho-

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mosexual men there was no temporal correlation between the presence of CMV viruria and serum IgM antibody titers. CMV IgM antibody was detected in a significantly higher ($p < 0.05$) proportion of serum samples from the 206 initially seropositive men (67% of 1,136 samples) than in the postconversion samples obtained from the 22 "seroconverters" (52% of 86 samples).

The higher prevalence of CMV IgM antibody in long-standing seropositive men than in recent seroconverters suggests that the former group is continually being reexposed to (and possibly reinfected with) exogenous strains of the virus. We believe that this intense exposure to CMV may lead to multiple infections with differing strains of "wild type" CMV.

To determine whether more than one CMV infection actually occurs, we analyzed multiple virus isolates from autopsy tissues of each of four AIDS patients by use of Southern blot hybridization to compare the genetic relatedness of clinical isolates of CMV (4). Each of the four patients we studied had at least two different strains of CMV, as determined by Bam HI restriction site polymorphisms revealed by Southern blot analysis. Similar results were reported by Spector et al (5).

Thus, some reinfections with CMV do occur in patients with AIDS, but they may represent only terminal CMV superinfections in patients already severely immunocompromised. To determine whether CMV contributes to the pathogenesis of AIDS at an earlier stage, it is necessary to determine whether exogenous reinfection with CMV occurs in healthy homosexual men prior to the development of AIDS. We are pursuing this objective in a prospective study (6).

CMV Infection of Both B and T Lymphocytes May Enhance Subsequent Infection of Lymphocytes by AIDS-Related Retroviruses. Rice et al (7) have reported in vitro infection of human peripheral blood mononuclear cells by low-passage CMV isolated from patients with various clinical syndromes. Using monoclonal antibody technology, they were able to infect both T and B lymphocytes, natural killer (NK) cells, and monocytes. Virus expression was limited to the synthesis of immediate-early CMV polypeptides; viral replication did not occur. "Wild" virus strains were more infective than laboratory-adapted viruses, suggesting that the latter are highly adapted to fibroblasts, whereas in "nature" CMV may be more lymphotropic. Immunocompetent mononuclear cells infected by CMV had diminished mitogen and antigen responses. These results correlate with previous observations of decreased lymphocyte proliferation responses to mitogens and antigens in patients with acute CMV infection (8–10). Such patients also have a transient defect in cytotoxic lymphocyte responses to allogeneic cells.

We have investigated the relationship between CMV serologic status and T-cell imbalance in homosexual men because we reasoned that if persistently abnormal ratios of helper:suppressor lymphocytes were found in homosexual men who lacked CMV antibody, some agent other than CMV must be related to their lymphocyte abnormality.

Helper:suppressor ratios ≤ 1.0 were found in 2 of 42 homosexual men without antibodies to CMV, versus 33 of 67 (49%) homosexual men with antibodies to the virus ($p < 0.001$). In order to determine whether asymptomatic primary CMV infection in homosexual men induces immunologic abnormalities, 34 of the 42 cytomegalovirus-seronegative men were enrolled in a prospective study to document the time of seroconversion and relate it to helper:suppressor ratio results. During an average follow-up period of 13.6 months, 12 (35%) seroconverted; 10 of these persons were asymptomatic. All 12 had helper:suppressor ratios ≥ 1.0 in the month prior to seroconversion, and all ratios dropped below 1.0 within one to three months following seroconversion (average nadir, 0.62). Helper:suppressor ratios remained ≤ 1.0 for an average of 9.6 months, and of the 7 seroconverters who were followed for at least twelve months, 3 maintained a ratio <1.0. The degree of abnormality of helper:suppressor ratio did not differ in the asymptomatic versus the symptomatic patients. Of 20 seronegative men whose initial helper:suppressor ratio was >1.0 and who remained seronegative, only 1 developed a helper:suppressor ratio consistently below 1.0. None of the men in this prospective study developed antibodies to the AIDS-associated retrovirus.

Our results suggest that cytomegalovirus is responsible for at least some of the immunologic abnormalities occurring in homosexual men. Even asymptomatic CMV infection induces profound disturbances in the ratio of helper to suppressor lymphocytes that may persist for prolonged periods. These abnormalities appear to occur prior to, and independent of, infection by the AIDS-associated retrovirus. As mentioned above, true reinfection with differing strains of CMV may occur; if so, repeat episodes of immunologic abnormality may be induced. Evidence for this is being pursued in our ongoing prospective study.

In summary, we suspect that cytomegalovirus infection is an important cofactor in the genesis of AIDS and that cytomegalovirus infection may be a necessary precursor for the subsequent full expression of infection by the AIDS-associated retrovirus (11). Indeed, the timing of these two infections may be critical; fully developed AIDS may occur only when retrovirus infection is superimposed on active cytomegalovirus infection (see below). We suspect that CMV infection must precede retrovirus infection, rather than the converse, because in San Francisco homosexual men a high rate of CMV infection preceded the onset of retrovirus seropositivity (Fig) (12).

CMV Infection Is Immunosuppressive and Causes Abnormal T-Cell Subset Ratios. CMV is known to cause T-cell dysfunction during acute infectious mononucleosis-like infection. Suppression of T-helper lymphocytes and mitogen responses occur and then revert to normal after convalescence (13, 14). A review of the immunologic consequences of acute CMV mononucleosis was published in 1984 (15).

CMV as a Cofactor in Kaposi's Sarcoma

In the United States, over 3,000 cases of malignant Kaposi's sarcoma (KS) have been reported since the beginning of the AIDS epidemic. There is increasing evidence that in a suitably predisposed (immunocompromised) host, CMV is capable of causing oncogenic transformation of endothelial cells leading to KS. This conclusion is based on four lines of evidence:

Oncogenic Potential of CMV. CMV has been considered a possible oncogenic virus by virtue of its ability to stimulate synthesis of DNA and RNA in host cells (16) and its ability to transform human embryo fibroblasts, which then grow as sarcomatous tumors when transplanted to immunosuppressed mice (17). Nelson et al (18) refined the latter observation by identifying the transforming segment of the CMV genome.

Serologic Evidence. The second line of evidence linking CMV to KS stems from work previously reported by Giraldo et al (19). A significantly higher prevalence of CMV antibody was detected in 46 elderly European and U.S. patients with KS than in two age-matched control groups without KS. This serologic difference was not found with antibodies to Epstein-Barr (EBV), herpes simplex (HSV), and varicella-zoster viruses.

Direct Evidence in Biopsies. Further evidence for an association of CMV with KS derives from the demonstration of CMV nucleic acids and antigens in KS tumor biopsies. Boldogh et al (20) detected CMV DNA in 6 of 12, and RNA in 3 of 9 KS biopsies from African patients. EBV and HSV type 2 sequences were not detected. We reported the presence of CMV RNA by in situ hybridization in 2 of 3 KS biopsies from homosexual men whose tumor cultures were negative for replicating CMV (21). Fenoglio et al (22) confirmed our results by detection of CMV RNA in a KS biopsy from a single homosexual man. Boldogh et al (20) also reported the presence of CMV nuclear antigen by anticomplement immunofluorescence in 14 of 16 KS biopsies from African patients. We have reported detection of CMV antigen by a similar procedure in 6 of 9 biopsy specimens from homosexual men (21), and more recently have found this antigen in an additional 9 of 18 cases examined.

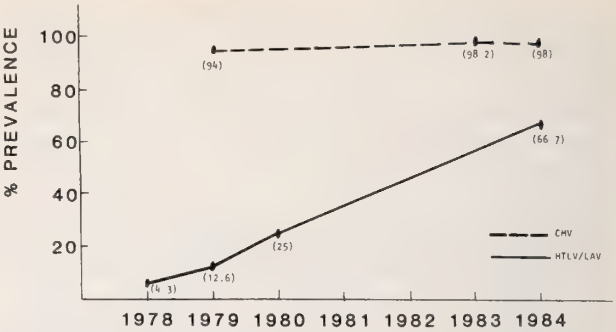

Fig. CMV and HTLV-III/LAV antibody prevalence in homosexual men attending the San Francisco Sexually-Transmitted Diseases Clinic. Adapted from Des Jarlais DC, et al, MMWR 1984; 33:377–379, and Drew WL, et al. J Infect Dis 1981; 143:188–192.

All these biopsies were negative for CMV by culture, indicating that CMV genome is present in a nonreplicative—and hence more likely oncogenic—state. Moreover, biopsies of normal skin from all but 1 of 22 of these patients were negative for CMV antigen, suggesting that the positive biopsies were not simply the result of disseminated CMV infection or a tropism of the virus for skin tissue.

These data do not prove a causal relationship between CMV and KS. Indeed, the presence of the virus or its gene products may only reflect reactivation of latent CMV in tumor tissue, or an affinity of CMV for neoplastic tissue, or carriage of the virus as a passenger within tumor cells. However, detection of CMV RNA and antigen suggests that the virus is not merely a dormant passenger but is actually directing the synthesis of viral protein(s).

Epidemiologic Evidence. The epidemiologic evidence for CMV involvement in KS derives from the heightened prevalence of this tumor in two groups of individuals, renal transplant patients and homosexual men with AIDS, who share the twin features of immunosuppression and extremely high rates of active CMV infection. There have been at least 36 reports of KS in renal transplant patients (23–26); it is estimated that KS accounts for more than 3% of all malignancies occurring in organ transplant recipients (24). Although CMV studies have not been specifically reported in these patients, renal transplant recipients as a group are subject to extremely high rates of primary or reactivated CMV infection (27), and all these patients are, of course, immunosuppressed during the posttransplant period. KS appears an average of 16–36 months after transplantation, suggesting an approximate incubation period for the development of the tumor in immunocompromised patients.

To date, we have studied 57 homosexual men with KS for CMV antibody, and all are positive. Of 39 who have had appropriate secretion cultures, 24 (62%) have been positive. Zur Hausen (28) has stated, "It is likely that the epigenetic burden of persisting viral DNA increases with the number of infections and is therefore correlated with age and exposure." In light of this, it is striking that the average age of homosexual men with KS is 36 years, and that they have been homosexual for an average of 17 years (29). A quantitative estimate of the extent of CMV exposure among homosexual men can be inferred from the finding that, even on a single sample, the semen of 25% of homosexual men is CMV-culture-positive (2). The high geometric mean titer of CMV antibody in homosexual men versus heterosexual controls, and the frequent finding of CMV IgM antibody in sera from homosexual men, also support the concept of frequent reexposure.

An important epidemiologic clue linking CMV to KS has come to light during the current AIDS epidemic—the marked discrepancy in the incidence of KS cases among homosexual versus heterosexual AIDS patients. In New York City as of 1982, KS accounted for 45% of the AIDS cases in homosexual men, but only 8% of AIDS in heterosexual men (29). This striking discrepancy suggests that a cofactor may be present in homosexual men that is not present in heterosexuals. This cofactor may be CMV infection, since intravenous-drug users, most of whom are heterosexual, do not have high rates of CMV infection. Of 143 intravenous-drug users in San Francisco and New York City studied in 1984, only 92 (64%) were seropositive, a rate similar to that in the general population (30). Other factors, including genetic (HLA-DR5 haplotype, Italian ancestry), may contribute to the disproportionate number of KS cases among homosexual men, but do not provide a total explanation.

The development of Kaposi's sarcoma in homosexual men is clearly a complication of their immunocompromise. Indeed, whether or not CMV is a cofactor in the pathogenesis of AIDS, we feel that the evidence is compelling that the virus makes a substantial contribution to the development of Kaposi's sarcoma. Definitive proof of an etiologic role for CMV in the genesis of KS would require the demonstration of protection by a vaccine.

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Malignant Lymphomas, AIDS, and the Pathogenic Role of Epstein-Barr Virus

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The etiology of acquired immune deficiency syndrome (AIDS) is not fully elucidated, but several human T-lymphotropic retroviruses have been considered as possible agents, with HTLV-III as the most likely offender (1–4). The vast majority of AIDS-related lymphomas are of B-cell origin, and the agent most frequently associated with B-cell neoplasm is Epstein-Barr virus (EBV). This article reviews the role of EBV in AIDS-associated lymphomas and the characteristics of these neoplasms. It concludes with new insight into possible immunomanipulations through thymus transplantation.

EBV in AIDS Lymphomas

Ubiquitous Viruses in Secondary Malignancies. Launched by Ehrlich (5), the concept of immunosurveillance against cancer was reactivated by Thomas (6) and then developed by Burnet (7), both of whom raised the possibility that cellular immunity is instrumental in destroying neoplastic cells. According to this concept, cancer cells arise continuously, and tumor-specific surface antigens are recognized as nonself with the elimination of the potential malignant clone by T cells. T-cell immunodeficiency will therefore predispose to a variety of malignancies.

Under certain conditions, ubiquitous viruses may induce target-cell proliferation leading to specific malignancies without necessary integration into the host genome. According to Purtilo and Linder (8) such viruses are characterized by (a) specific cellular targets exhibiting viral receptors; (b) ability of the virus to act in part as a mitogen (therefore, not necessarily as an oncogen) of the target cell; and (c) induction by the virus of target-cell proliferation rather than lysis. In addition, the host must display an immune defect which will allow gradual cell proliferation, and must survive for months. Finally, a conversion of these malignancies from polyclonality to monoclonality may occur, due to specific cytogenetic rearrangements allowing activation of oncogenes in the target cell. Occasionally, proliferation during the polyclonal phase is reversible, when viral burden is decreased or the immune competence restored.

The association between ubiquitous viruses and human malignant tumors is better understood in relation to EBV.

Normal Immune Responses to EBV. EBV, the etiologic agent of infectious mononucleosis (IM) (9) and, most likely, of African Burkitt's lymphoma (10) and nasopharyngeal carcinoma (11), is a ubiquitous herpes virus of humans and several simian primates. It exhibits B-cell tropism with an extraordinarily restricted host range, determined at the C3d receptor level (12).

In IM the virus infects many different B lymphocytes. IM is therefore called a polyclonal B-cell disease. The potential life-threatening polyclonal B-cell proliferation, reflected during the first week of IM infection by hypergammaglobulinemia, is counteracted by the activation of adequate effectors, that is, interferon and natural killer (NK) cells (13–15). Probably the most powerful protective mechanism is activation of suppression/cytotoxic T cells (Ts) during acute EBV infection (16). The atypical lymphocytes in the blood during IM are predominantly Ts cells that recognize the virus-induced membrane antigen

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on the surface of infected and transformed (immortalized) B cells. As early as the second week of IM, T cells are found to suppress B-cell activation, as measured by immunoglobulin induction in vitro (17).

The subset of Ts (killer) cells participating in this interaction is not necessarily specific for EBV-induced B-cell activation, since the same T population also suppresses the pokeweed-mitogen-induced response of autologous and allogeneic B cells (18). This may explain why EBV-specific T-cell memory during the disease develops slowly compared to most antibody responses to EBV-determined antigens (19). In 1981 EBV-specific Ts cells were shown to recognize the virus-induced membrane antigen on the surface of target cells in association with HLA-A and -B antigens and possibly -C, which represents a polymorphic restriction governing this specific toxicity (20). Since virtually all healthy EBV-seropositive donors have a high frequency of virus-specific memory T cells, the specific T-cell-mediated cytotoxicity may have an important role in controlling the persistent viral infection of the carrier state.

The T lymphocytes attack the EBV-transformed cells, liberate the antigenic material necessary for the late emergence of antibodies to nuclear-associated antigen (EBNA), and limit the clinical infection with EBV without completely eradicating it. For each 10^7-10^8 circulating lymphocytes, at least one cell may harbor EBV genomes at any given time (21).

Humoral immunity responds to EBV-specific antigens in a predictable time-dependent course (22). Immunoglobulin (Ig)M antiviral capsid antigen (VCA) appears initially during the production of viral particles with simultaneous formation of antiearly antigen (EA), usually the diffuse component (D). IgG anti-VCA will subsequently substitute for IgM anti-VCA. EBNA arises generally later, weeks or even months after the onset of illness (22–24). EBNA with IgG anti-VCA persists lifelong and indicates the latency of the virus. The antibody responses in silent primary EBV infections of infants less than two years old differ from those seen in IM only in that antibodies to the early EA complex are directed against restricted EA component (R) instead of D and in that heterophil antibodies do not rise to significant titers (24).

Lymphomas in Immunosuppressed Patients. Lymphomas arising in immunosuppressed patients may be shown to exhibit EBV material in several ways: first, EBNA can be detected in the tumor by immunofluorescence, and second, cloned fragments of EBV genome can be used to examine tissues for the presence of EBV DNA, either by nucleic acid hybridization of cell spots from which the DNA has been extracted in situ, or by hybridization of DNA that has been transferred to nitrocellulose by Southern blotting (25).

Using these techniques, EBV or its genome was detected in hyperplastic lymph nodes and in lymphomatous growths in transplant recipients (26). EBV-associated lymphoid neoplasms have been described in recipients of renal and thymic transplants in whom the lesions have been thought to be polyclonal in origin because different immunoglobulins were found within individual tumors (27–29). Biopsies of these tumors performed later in the clinical course have shown restriction of cellular immunoglobulins to single light-chain and heavy-chain classes, consistent with the evolution of monoclonal lymphomas (30). In at least one patient with severe combined immunodeficiency after bone marrow transplantation, analysis of cellular immunoglobulin and immunoglobulin gene DNA from different tumor masses indicated both monoclonal and oligoclonal B-cell proliferations (31). It is apparent in view of these data that EBV-induced polyclonal activation of B cells can evolve to oligoclonal and finally to monoclonal B-cell lymphoma.

EBV Infection in AIDS Patients. Evidence is ample of EBV infection in patients with AIDS as well as in healthy homosexuals. In over 300 homosexual men from New York and Omaha, EBV seropositivity was 91% in controls, 98% in healthy Omaha homosexual men, and 100% in homosexual men from New York (32, 33). Antibody to EA was twice as frequent (33%) in homosexuals with AIDS and persistent generalized lymphadenopathy than in heterosexual controls (14%) and was directed mainly to R component (34). Geometric mean titers of IgG anti-VCA and anti-EBNA were increased in homosexual men, and their EBV-receptor-bearing lymphocytes also showed an enhanced antibody response to EBV antigens; there was also an increased load of EBV in oropharyngeal secretions and peripheral blood leukocytes.

We have been able to demonstrate similar high titers of anti-VCA in 21 homosexuals with and without Kaposi's sarcoma: titers in excess of 640 were the rule, and in one patient the titer exceeded 1:40,000 (measured by indirect fluorescence) (35). There is also evidence that in AIDS patients, simultaneous infection with more than one strain of EBV is possible (36). The existence of high anti-VCA and anti-EBNA titers suggests that in homosexual men with AIDS, the EBV infection antedates the appearance of AIDS. In general, considering other states of immunodeficiency (Hodgkin's disease, ataxia telangiectasia, X-linked lymphoproliferative syndrome, renal and bone marrow allograft recipients), antibody titers to the virus will be unusually low when immunodeficiency antedates primary infection with EBV (24, 37, 38) and high when immunodeficiency follows infection (24, 29, 39).

In the homosexual and AIDS populations, EBV infection is associated with another herpetic infection, cytomegalovirus (CMV). The prevalence of antibody was 96% to CMV and 94% to EBV in a group of 89 sexually active homosexuals (40). The CMV antibody titers positively correlated with the T-helper (Th)/Ts ratio, number of partners and venereal disease episodes, number of Ts cells, and EBV titers. CMV infection in itself can produce prolonged alteration in cell-mediated immunity both as a primary and as a reactivated infection (41–43). A possible relationship between EBV and CMV, resulting in more profound immune deficiency in patients with AIDS, has been suggested (44).

Polyclonal Activation of B Cells in Homosexual Men. Patients with AIDS express marked abnormalities of B-cell activation and immunoregulation characterized by: (a) lack of resting B cells, which are stimulated by the T-cell-independent *Staphylococcus aureus* Cowan strain I (SAC) mitogen (45); (b) decreased numbers of partially activated cells, which can differentiate into antibody-secreting cells under the influence of pokeweed mitogen when provided with adequate numbers of Th; and (c) increased (10×) numbers of fully differentiated B cells, capable of spontaneously secreting antibodies (46–48). This polyclonal B-cell activation is probably responsible for the elevated levels of serum immunoglobulins (49, 50), the autoimmune thrombocytopenia described in homosexual men (51), and the elevated levels of circulating immune complexes (52). The EBV-induced polyclonal B activation occurring in the lymph nodes may also be responsible for the development of persistent generalized lymphadenopathy in AIDS patients and probably precedes the appearance of true lymphomas. In 1983, for all cases reported by Lane et al, the patients were culture-positive for EBV and CMV (45). Alternative possibilities exist. Among them are the T-cell-independent transformation of B lymphocytes by retroviruses (53) and the elaboration of B-cell growth factor and differentiation factors by T cells transformed with HTLV-III (54). The evidence for this hypothesis, however, is meager.

Persistent Generalized Lympadenopathy in AIDS

A syndrome of persistent generalized lymphadenopathy (PGL) has been noted in homosexual men and patients receiving transfusions of blood or blood products (55–57). The homosexuals with PGL differ from homosexuals lacking lymph node enlargement in that the former have higher numbers of circulating lymphocytes, a greater number of Ts (T8+ or Leu 2+) lymphocytes and NK cells (Leu 7+) (58), and more severely depressed lymphocyte mitogenic responses (57). The immunologic injury is, however, not initially fully expressed, since some investigators have described NK activity in PGL patients as well as normal IL-2 production (59).

Initial West Coast reports of PGL failed to show progression toward a more malignant form of AIDS or neoplastic transformation (60, 61). An increased (15%–20%) incidence of progression from benign PGL was first reported by New York investigators (62). The difference may be explained by a 1-year lag time in the West Coast epidemic and the heterogeneity of the study groups evaluated. On the East Coast, the histology of the lymph nodes showed three nondiagnostic patterns (62): (a) follicular hyperplasia (63, 64), reminiscent of the early stage of IM (65, 66), (b) follicular involution with expansion of the paracortical area, and (c) a mixed pattern of follicular hyperplasia and follicular involution in the same lymph node. These changes resemble those seen in viral lymphadenitis, in X-linked lymphoproliferative disorders (67, 68), and in renal transplant recipients (28, 69), conditions known to be associated with EBV infection. The EBV genome was demonstrated in lymph nodes from 3 of 4 individuals with AIDS and PGL (8).

The polyclonal B-cell proliferation of PGL is documented by immunofluorescent and immunoperoxidase staining showing mixed populations of lymphoid cells expressing all classes of heavy- and light-chain immunoglobulins in various proportions (64), with cells with both κ and λ markers present in the germinal centers, deep cortex, and medulla (64, 70). On occasion, monoclonal surface immunoglobulin patterns were described, probably as an expression of a prelymphomatous state (71). The T cells from the interfollicular areas of PGL phenotypically express the Ts marker, whereas in normal lymph nodes they express the Th (Leu 3+ or T4+ phenotype) (70). Most likely, the normal lymph node Th cells are destroyed by HTLV-III: the residual or reactive Ts cells were shown to be strongly reactive to anti-HTLV antibodies (70). Interestingly, of the patients who did develop malignant complications, most had a pattern of follicular involution prior to diagnosis. The pattern did not differentiate between lymphomas or Kaposi's sarcoma (71). A similar involution was also seen when PGL progressed toward AIDS with opportunistic infections (71).

Clinically, PGL may precede or coexist with malignant lymphoma; only aggressive simultaneous/sequential lymph node biopsies of suspected lymph nodes may help to determine the true incidence of early lymphomatous transformation (64). Gallium scans are useful in deciding which node(s) to rebiopsy (72).

Non-Hodgkin's Lymphoma

A true increase (1.4-fold) in the relative frequency of aggressive non-Hodgkin's lymphoma (NHL) was described in 1984 in the male population not at recognizable risk of developing AIDS (73). Ziegler et al examined the Cancer Registry for the five-county San Francisco Bay area (74), finding no cases of high-grade NHL among single men aged 20–50 in the period 1975–1978, but 9 cases in the period 1979–1982 ($p < 0.001$ by chi square). This ninefold increase in an area at increased risk for AIDS could not be due to a ninefold influx of single men aged 20–50 in the Bay area, but reflected rather the increased incidence of high-grade lymphoma in homosexual men. A similar study in Los Angeles County found that the number of NHL in single men—when compared to findings in single women, married men, and married women—had increased for the year 1981–1982 from 1% of all NHL to 16% of all NHL (over 1973–74). The incidence of all forms of lymphoma was about 3%–4% in over 3,000 patients with AIDS reported to the Centers for Disease Control in 1983 (75).

NHL in AIDS patients has several unusual pathological and clinical features, including (a) B-cell origin of high-grade type; (b) association with EBV and HTLV-III; (c) prodromal manifestations; (d) presentation at advanced stages; (e) high frequency of extranodal (bone marrow, gastrointestinal, liver) and primary brain involvement; (f) poor prognosis.

B-Cell Origin. In the well-known series of 90 homosexual men with NHL studied by Ziegler et al (76), the histologic subtypes in the majority of cases were consistent with a B-cell or pre-B-cell origin, although cell-marker studies were not performed systematically (77). Previous and subsequent reports confirmed this finding (74, 78–83). When examined, almost without exception light-chain staining confirmed the monoclonality of the B-cell lymphomas (79, 80). Thirty-two of 35 lymphomas studied by Ziegler et al (76) and 21 of 28 NHL studies by Levine et al (79) were reported to display monoclonal surface immunoglobulin. In the absence of adequate κ/λ markers, the newer technique of heavy-chain immunoglobulin genes rearrangement may provide the proof for B-cell origin (84, 85).

Association with EBV and HTLV. PGL, which may be a prelymphomatous state, is associated with HTLV-III infection, as documented by the expression of HTLV-III antigens in interfollicular T cells in the "reactive" lymph nodes (70). In NHL, the nodes have reduced T-cell infiltration, probably due to T-cell destruction by HTLV-III; in a 1984 study of 14 patients with NHL and AIDS, 11 tumors were positive for anti-HTLV-III antibody tested with ELISA (79). Although HTLV-III or related HTL viruses can infect B cells from humans and primates in vitro (53, 86), this did not appear to occur in vivo (84). HTLV-III cytopathic effect on T cells in AIDS may provide just the immunologic milieu for development of EBV-associated lymphoma. Southern blot analysis of DNA from NHL lymphomas occurring in homosexual men revealed integrated sequences related to EBV but not to HTLV-III (84, 87). Two of the four Burkitt's-like lymphomas studied by Ziegler et al were found to stain positively for EBNA (88).

Grade of Malignancy in AIDS. The New Working Formulation of NHL provides a unifying concept for the otherwise diverse and confusing range of classification schemes. According to this formulation, modified as suggested from the Cologne study, lymphomas of high-grade malignancy (ML) comprise (89, 90) Class G (ML, diffuse, large cell cleaved and noncleaved, immunoblastic), Class H (ML, diffuse, lymphoblastic convoluted and nonconvoluted), and Class I (ML, Burkitt's type—small, noncleaved).

In a 1984 series, (90 NHL in homosexual men), Ziegler et al found 56 lymphomas (62%) to be high grade: small, noncleaved cell (Burkitt or Burkitt-like), 32; large cell, immunoblastic, 22; lymphoblastic, 2 (76). Seventeen of 18 cases reported by the same author from the San Francisco Bay Area were also high-grade (74), as were most of the 22 NHL cases described by Ioachim et al at a single New York institution (91). It is of significance that the high-grade malignancy NHL in AIDS patients represented from 60% to

95% of all histologic types, whereas only 44% of the NHL developing in the general population were high grade (90). NHL of high-grade malignancy are overwhelmingly of B-cell phenotype in the general population as well (92). An important difference between NHL of high grade in the general population and in patients with AIDS may be related to the T-lymphocyte infiltration in the lymph nodes involved by tumor. The median percentage of T lymphocytes in cell suspensions from 26 B-cell lymphomas arising in a non-AIDS population was 31% (92), most were Th phenotype and correlated with better prognosis. Patients who achieved complete remission had higher levels of T cells infiltrating the lymphomatous nodes, whereas patients who died had low numbers of infiltrating T cells. On the other hand, T cells present in the B-NHL lymphomas from patients with AIDS seemed to be quantitatively reduced (93), a fact not suprising in view of the Th tropism of HTLV.

Burkitt's Lymphoma in AIDS as Prototype. The outbreak of Burkitt-like lymphoma in homosexual men was first reported by Ziegler et al in 1982 (88). Four cases of undifferentiated monoclonal B-cell tumors were seen in a 9-month period in San Francisco. Two of these tumors contained EBNA, one of them had CMV antigen, and a third contained no viral antigen, but EBNA and CMV antigens were detected in the overlying epithelium. Two patients had intraoral tumors, and a third had tumors almost exclusively in the central nervous system (CNS). Doll and List almost simultaneously reported a similar case in a homosexual man in Tuscon, Arizona (mesenteric mass, no bone marrow, liver, or CNS involvement) (94). Since then, numerous reports have described similar Burkitt-type tumors in patients with AIDS (78, 79, 81–83). The same Burkitt's pattern has been documented in several hemophiliac patients, with both bone marrow involvement and evidence of EBV infection (95, 96). The risk of Burkitt-like NHL is understandably high in hemophiliacs; in a 1983 study, 70% had serologic patterns consistent with persistent or reactivated EBV infection (anti-VCA ≥ 1:320 or anti-EA ≥ 1:40), whereas only 25% of adult controls had similar antibody patterns (97).

Specific Translocations of Burkitt-like Lymphoma in AIDS

Endemic as well as nonendemic Burkitt's lymphoma are characterized by specific translocations that affect chromosomes 2, 8, 14, and 22 (98–101). The most frequent of these translocations is t(8;14)(q24;q32), while the variant translocations t(2;8)(p12;q24) and t(8;22)(q24;q11) seem to occur less frequently (101). Chaganti et al were the first to report specific translocations in Burkitt-like lymphoma in AIDS (102). Both patients described had EBV anti-VCA titers. One of the patients expressed the t(8;14) translocation and had κ light-chains on the tumor cell surface; the second expressed the t(8;22) translocation and had λ chains. This pattern of expression was consistent with the location of the determinants for light-chain to chromosome 22 (103). It is significant that the cellular oncogene c-myc is located on chromosome 8, where its break occurs in the Burkitt's lymphoma translocation (8q24) (104); the c-myc gene becomes translocated to chromosome 14 in the t(8;14) translocation (105) and is subsequently placed under the transcriptional control of the determinants for immunoglobulin heavy-chains, mapped to chromosome 14 (104).

The role of EBV infection may be limited to polyclonal B-cell activation, unsuppressed by the deficient T-cell surveillance; the specific translocations described would occur as an expression of a genetic or acquired (viral?) instability. The role of viruses has been suggested by a 1979 study of ataxia-telangiectasia lymphoid cells, which developed an 8:14 translocation identical to that seen in Burkitt's lymphoma after in vitro infection with EBV (107), a phenomenon not observed after EBV infection of lymphocytes from normal persons. Indeed, three cell lines derived from a homosexual patient with AIDS and Burkitt's lymphoma were shown to produce an unusual strain of EBV which both transformed cord blood lymphocytes and induced EA in Raji cells (108). A t(8;12) translocation occurred in all three lines, but the cells synthesized immunoglobulin M with κ chain in contrast to the usual concordance between a translocation involving chromosome 22 and λ chain synthesis. Therefore, either that translocation may have occurred as a separate event from immunoglobulin gene rearrangement, or the postulated sequence of immunoglobulin gene arrangements is not always adhered to.

Since the initial report of Chaganti et al, the same translocation t(8;14)(q24;q32) has been described in at least seven more AIDS-related Burkitt's-like lymphomas (80, 81, 109, 110). All of these had high EBV titers, and in one case the EBV genome was demonstrated by molecular hybridization in the tumor mass (88, 110). When NHL developing in non-AIDS patients are analyzed, abnormalities of the clonal chromosomes can be detected in 95% (42 of 44) of cases, using high-resolution techniques (111). At least half of the patients with NHL have translocations involving chromosome 14, but unlike Burkitt's lymphoma, no single donor chromosome has been regularly implicated (112–115). The translocation t(8;14) carries the poorest prognosis (12 months survival) (115).

Associated CNS Disease. Thirty-eight of the 90 patients reported by Ziegler et al (76) had evidence of CNS metastases, either as a brain mass or involving the meninges, cranial/peripheral nerves, or paraspinal areas (76). The incidence of CNS involvement is far in excess of that expected in de novo NHL lymphoma (90) and is similar to that of lymphomas associated with congenital immunodeficiency (116), autoimmune disease, renal transplant recipients (117), and Hodgkin's disease (118). Primary CNS lymphoma, usually 1.6% of all cases of extranodal NHL (119), is much more frequent in AIDS patients (120–122). Immunoglobulin marker studies revealed monoclonal B-cell marking in four of five tumors studied in 1975, all of high grade (122).

The CNS may represent a favorable site for primary B-cell lymphomas associated with immunodeficiency because of immunologic tolerance within the brain (123). For example, the incidence of primary DHL of the CNS in renal transplantation is 350 times that of the normal population at large (124). Hochberg et al found serologic evidence in 5 patients with primary CNS lymphoma of a recent primary infection with EBV; DNA preparations from tumor tissue in 1 patient but not from adjacent normal brain tissue contained the EBV genome (125). The prognosis associated with CNS lymphoma associated with AIDS is uniformly poor, with failure of treatment or recurrence in the original treatment site or fatal opportunistic infections. Median survival in the case of non-AIDS primary NHL of the brain was 15 months with surgery and radiation therapy alone (126) or 44 months for patients also receiving chemotherapy (127). For AIDS-associated CNS lymphoma, the medial survival is about 2.5 months (88, 122).

Other Lymphoid Neoplasms

There are several reports of Hodgkin's disease arising in patients with AIDS (128–131). When specified, the histologic types were either of mixed cellularity or nodular sclerosis. Most cases had prodromal persistant generalized lymphadenopathy and were either stage III or IV at the time of diagnosis. Thirteen of the 16 cases had subdiaphragmatic involvement, and in 1 patient the spleen seemed free of disease while marrow and liver were involved (131). Most patients died in less than 4 months, but 3 of 16 lived longer than 1 year.

We described the first case of a T-lymphoblastic neoplasia at the preleukemic stage in a patient with AIDS and Kaposi's sarcoma (132). The marrow was involved by a terminal transferase positive population of blasts, hyperdiploid when examined by flow cytometry. To the best of our knowledge, no additional reports of T-cell malignancies in AIDS patients have appeared.

Teatment of AIDS-Related Lymphomas

In the series compiled by Ziegler et al (76), 66 of 90 patients were evaluable for treatment response and survival. Standard combination chemotherapy or radiotherapy or both were given. Complete responses were achieved in 35 patients (53%), but within 2 years 54% had relapsed. In the high-grade NHL, median actuarial survival was 6 months, or 50% shorter than in non-AIDS patients with comparable tumors (91). AIDS-associated NHL of intermediate grade had a median survival of only 5 months, compared with 30 months in non-AIDS. Death was caused by either lymphoma or opportunistic infections. Small series tend to project a better outlook: a complete remission rate of 100% in 9 high-grade NHL was reported by Ziegler et al (74), and complete remission in 4 patients of 6 surviving between 5+ and 3+ months was reported by Riggs et al (87); none of these 4 patients had opportunistic infections or CNS involvement. In general, malignant lymphomas in AIDS patients should be treated aggressively with combination chemotherapy with regimens acceptable for therapy of the specific lymphoma type in non-AIDS patients (133–136).

Vigorous cytoxic treatment may increase the propensity for opportunistic infections, but at present choice is limited. The optimal approach to primary CNS lymphoma has not yet been defined. Radiation therapy to the brain has had little success, and the characteristics of the dose-response curve, optimal time-dose relationship, and use of total neuraxis radiation are uncertain; whole-brain radiation to a dose of at least 40 GY over 4–5 weeks is reasonable (137). Experience with chemotherapy is limited, but clear responses have been reported to high-dose methotrexate (138) and to a combination of cyclophosphamide, doxorubicin, vincristine, and dexamethasone (139).

Thymus Transplantation for Treatment of AIDS

Even if combination chemotherapy induced complete remission of lymphoma in AIDS patients, the underlying immunodeficiency is not improved and may decline further with potent chemotherapy. An effective anti-HTLV-III treatment will not reverse the immunosuppression and may not eradicate the EBV infection. Acyclovir inhibits productive replication of EBV DNA by inhibiting the EBV-associated DNA polymerase (140), but does not alter the synthesis of the latent EBV genome (141). Normal hosts do not develop B-cell neoplasias, even if they are EBV carriers because of adequate T-cell function. It seems therefore logical to consider the immune reconstitution of AIDS patients, either with or without a history of NHL. Thymus transplantation with cultured thymus fragments (TCF) could conceivably offer the easiest reconstitutive approach, since it is devoid of infectious complications (a preparative in vivo regimen is never used) and it can be performed across the HLA barrier (142, 143). Transplantation with TCF could be justified by the existence of characteristic changes in the thymus of AIDS victims consistent with an organ-specific immune complex attack by polyclonal immunoglobulins directed against the thymic epithelial cells, Hassal's corpuscules, and certain subsets of thymocytes (144–146).

Dupuy et al have attempted immune reconstitution with cultured thymic epithelial cells in 14 adult patients with AIDS (147). They reported transient clinical improvement and absence of new opportunistic infections; partial immunoreconstitution was evidenced by an increase in peripheral blood lymphocytes (12 of 13 cases) as well as liver infiltration of T4 and T8-positive cells (5 of 7 cases).

We have reported the results of transplantation with TCF in 2 patients with AIDS and Kaposi's sarcoma (148). A transient benefit was noted in 1 patient, as evidenced by stabilization of Kaposi's lesions and improvement in the absolute T4 and T8 counts and T4/T8 ratio (from 0.31 to 0.84). This improvement lasted for only 6 weeks. The second patient did not improve either clinically or immunologically.

Failure of TCF transplants in the absence of curative antiviral (anti-HTLV-III) medication is most likely related to viral destruction of T4 cells in the perivascular/peri-Hassal areas of the thymus (148, 149) and to immune destruction of thymic epithelium by antibodies that may cross-react with antigenic determinants shared with HTLV-III (150). Once the elimination of HTLV-III is accomplished, transplantation with TCF may prove of significant benefit in permanently assuring the immunoreconstitution of AIDS victims.

Summary

EBV, an ancient and ubiquitous herpes virus of humans, is in symbiotic balance with its natural host species. Immunocompetent people as well as patients with AIDS have evidence of EBV infection close to 100%. EBV-transformed proliferating cells are normally inhibited by specifically sensitized HLA-restricted T cells. Because of T-cell defects, patients with AIDS are unable to restrain the polyclonal proliferation of latently infected B lymphocytes and most of them develop persistent generalized lymphadenopathy. A genetic or acquired (viral?) instability of EBV-transformed (or merely stimulated) B cells will permit clonal evolution with appearance of aggregates of lymphoma cells within the hyperplastic germinal centers of AIDS-associated PGL. The monoclonality of these lymphomas may be proven by either light-chain markers and/or heavy-chain Ig rearrangement; in the instance of Burkitt's lymphoma, it is also expressed as specific cytogenetic changes. When examined, a definite number of these B-cell lymphomas either stain positive for EBNA or exhibit the presence of EBV genome in hybridization studies, implicating EBV as a possible causative agent. Although standard aggressive chemotherapy of AIDS-associated malignant lymphomas may result in brief periods of remission, any successful treatment will have to address both the viral etiology of AIDS and the reconstruction of the immune system.

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Toward a Vaccine against AIDS: Rationale and Current Progress

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Human acquired immune deficiency syndrome (AIDS) is a progressing epidemic of high lethality. In the U.S. it is still confined primarily to identifiable high-risk groups, such as homosexual and bisexual males and intravenous drug abusers, but potential exists that from foci of heterosexual transmission, AIDS might emanate into the general population in a pattern closer to that of other sexually transmitted diseases. The syndrome is caused by a human cytopathic and exogenous retrovirus—HTLV-III/LAV (also known by other designations, including AIDS-related virus and, generically, human immunodeficiency virus). As such, a number of special principles apply to both interventive and preventive studies.

Based on the life cycle of retroviruses, the viral RNA makes a double-stranded DNA which integrates into cellular DNA, probably at random sites as proviral DNA. Additionally, this virus also maintains significant quantities of unintegrated viral DNA in the infected cell. Proviral DNA can be replicated with cellular DNA if the cell is not killed outright in the first cycle of infection. Importantly, in many retroviral systems the expression of proviral DNA is completely silent at the RNA and protein levels. In conjunction with the availability of immunologically protected sites, the potential of a silent residuum of proviral DNA implies that it may be difficult, if not impossible, to ascertain whether an individual once infected with a species-compatible retrovirus has successfully overcome and eliminated the invading virus. In people once infected with HTLV-III/LAV, whether they show any signs of disease or not, a persistence of antiviral antibody over time is obvious, and re-isolation of virus from peripheral blood cells is common.

From these known and implied facts we can define the population in which preventive vaccine measures would be primarily considered as previously uninfected individuals at risk for future exposure. Subsets of vaccine strategies, such as passive serotherapy, cannot be excluded for periinfection prophylaxis. However, the previously infected individual would seem to profit more from interventive measures aimed at diminishing the probability of progression to clinical AIDS (1–3).

Approaches to a Vaccine against AIDS

A large number of possibilities for an anti-HTLV-III/LAV vaccine exist today (reviewed 4, 5). They cover the spectrum of vaccine approaches (Table 1); some are immediately practical, others are still largely theoretical. The object is to narrow the number of choices down to the optimal current strategies.

Several initially attractive choices were not seriously considered for early attempts for human trials. These include killed-virus preparations and natural or genetically engineered nonpathogenic variants. The latter are very attractive because deletion of a specific gene, such as the trans-activator gene (*tat*) or its receptor, could make a noncytopathic virus which otherwise grows and expresses all other antigens (6).

However, killed retrovirus is known to be a notoriously poor inducer of immunity, so much so that kittens immunized with inactivated feline leukemia virus actually could do worse on challenge than the controls (7). A key feature of

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TABLE 1
Paths to a Vaccine to Prevent AIDS

1. Nonpathogenic infectious HTLV-III
2. Killed HTLV-III
3. Virus subunits
4. Genetically engineered subunit proteins
5. Synthetic peptides
6. Infectious recombinant viruses
7. Anti-idiotypes

retroviruses is that the long terminal repeats (LTRs), with their powerful molecular promoters and enhancers, are necessary for viral integration into cellular DNA. Even a truncated integration of LTRs themselves next to cellular genes could be deleterious. The classical "promoter insertion" hypothesis has shown that integration of chicken leukosis retroviral LTRs, either upstream or downstream from a cellular oncogene, could cause lymphoma at a later time (8). Prudence would dictate that viral nucleic acids of HTLV-III/LAV not be introduced in vaccine preparations if other equally feasible means are available.

The subunit approach, in which antigens of choice are derived from the virion or infected cells, is attractive only if technology exists for the large-scale purification of intact product. As an initial approach, the complexity and the natural placement of these antigens may make the subunit a useful learning process. The extensive glycosylation of some of the antigens of choice can cause significant folding changes of epitopes, even if minimal changes in sugar-chain addition are encountered (9).

Genetic engineering approaches have reached great sophistication; vector systems exist in bacteria, yeasts, and mammalian cells which could theoretically produce any complex antigen with great fidelity and with the necessary post-translational modifications. Export systems as well as superinducible built-in promoters could ensure large quantities of expressed and extended quasi-native antigen. This appears to be a major choice to be considered seriously.

A special synthetic subset of epitopes defined by subunit proteins can be defined by individual DNA-sequence-defined peptides which could have an enhancing effect, or even elicit protective immunity by themselves.

Two other approaches remain. One of these is the anti-idiotype as an internal image of the epitope defined by the topology of the reactive site of the second antibody. This would be a safe preparation, potentially of human origin, which could yield a broadly reactive response (10). However, a neutralizing or a protective monoclonal antibody would seem to be needed as the initial immunogen. Although these are not yet known to exist, the spectrum of technology for developing the correct monoclonal antibody is accessible.

The approach of infectious recombinant viruses holds great promise, and as defined below, has reaped some successes. In future we can consider a number of possible vector agents that would safely express HTLV-III gene products of interest and elicit a strong protective response. This approach has the additional advantage of affording a simple and tested delivery system under field conditions.

Choice of Immunogen and Presentation of Epitopes. Three areas of consideration have to be addressed. The first is that the wealth of genes of HTLV-III/LAV is unexpectedly large for a retrovirus, and that the function of several of these genes is not understood. Of the currently known viral gene products (Table 2), only some are important to viral growth and the induction of killing potential for T4 cells. Based on analogies with every other retroviral system, where in vivo protection from disease was achievable, the major antigen to consider is the large external glycoprotein defined by the 5′ end of the *env* gene, the gp120. The 3′ end of *env*, the gp41, defines the transmembrane protein (*tmp*) anchor for the gp120 and may additionally function in con-

TABLE 2
Viral Gene Products from HTLV III for Vaccine Consideration

Viral gene	Product size (kDa)	Function	Needed for growth and CPE	Immunoreactivity in humans
env 3′	gp41	Transmembrane anchor	Presumed, holds gp120	Pathognomonic
env 5′	p58-gp120	Attachment Penetration Targeting	Necessary	Early, strong
lor/3′ or (F)	p22-28	?	Not needed	yes
tat	p14-15	Fusion protein gene "rheostat"	Needed for significant growth	yes
sor(Q)	p23-25	?	Not needed	yes
pol	p51/p66	Reverse transcription	Key replicating enzyme	yes
gag-pol	p14	Protease frameshift protein	Need to cleave *gag* polyprotein	?
gag	p16 p24 p17	Core components	Key internal structures	Strong against p24 and p17

CPE, cytopathic effect.

ferring appropriate topology to the complex, as well as having additional properties. The *env* gene products are exterior on the virion and, as such, are first exposed to the immune system, whereas other antigens are interior and may not be seen by the various effector systems. Although this is probably true of the virion, infected cells may display different patterns of expression at the cell surface, and *gag* region modified precursor proteins have been described to be on the cell surface in some retroviral systems (11). Accordingly, antigens defined by genes critical for viral growth and cytopathogenicity which are interior cannot be excluded, especially in the context of antigenic processing which attends the induction of cell-mediated immunity. For example, humoral or cellular immunity to reverse transcriptase, *tat* proteins, some *gag* gene proteins, and especially the key cleaving protease may all be co-considered for future advantage.

The major *env* gene products are the antigens of choice based on current conventional wisdom. However, these are not simple proteins, because half the molecular weight of the gp120 is present as sugar residues. These carbohydrate moieties may be beneficial in ascertaining the correct folding of the protein backbone, or may be detrimental in preventing the attachment of antibody. Glycosylation can be studied either by removing the sugars from the formed molecules or by interfering with glycosylation via drugs during the synthetic process.

The antigen itself may be less than optimal if it is not presented properly. For example, the gp in both the mouse and the cat leukemic systems is much less effective if presented in pure form. The antigenicity of the complex is about tenfold more effective as a complex *gp-tmp* rosette, defined by the hydrophobic attraction of the internal *tmp* (12).

Thus, the availability of the correct antigen per se may not be enough in the absence of a proper presentation/adjuvant system. In considering future use in humans, alum is the only acceptable system in general use, although INDs may become available for alternate systems, such as muramyl-dipeptides, and other adjuvants. Very potent adjuvant systems exist, such as immune stimulating complexes (ISCOMS) derived with quil A, which raise the relative stoichiometry of antigenic response by several orders of magnitude (13). Initial approaches in the experimental HTLV-III/LAV system are mentioned below.

Problems to Consider. A number of significant problems are anticipated in the development of a vaccine against AIDS. Most is known about the obvious genetic heterogeneity among virons isolated (14). Some appear divergent by as much as 20% of amino acid sequences in the most variable gp120 region, as predicted by DNA homology and by actual two-dimensional oligopeptide maps. Current strains of virus also appear to contain more than one species as defined by multiple proviral DNAs in infected cells. The variability and the complexity of inocula in nature are uncertain as well, because of possible genotypic and phenotypic mixing known to exist in some retroviral systems. It is possible that mutations are occurring constantly in nature under immunologic pressure, as with some lentiviruses. However, in culture, a given gp120 appears to be stable with time and even with passage to other human T cell lines.

The key question is whether an antigen, for example gp120, from a single virus type would eventually induce antibodies which neutralize less related variants. Although in some classes of virus, cross-protection has not been described, in several families of retroviruses, group-specific and even interspecies-specific neutralizing antibodies have been induced by repeated immunization based on conserved epitopes (15).

Several additional questions have to be considered. Although a primary focus will be to induce neutralizing antibodies with gp120, it may be that antigens other than gp120 itself, if these are also involved in cell fusion, may be equally important as immunogens to obtain adequate protection. The question of protection is not easy to answer because there is no exact model for the induction of disease (see below). As other antigens are assessed for immunogenicity, one has to remember that with some retroviruses, the *tmp* (gp41 equivalent) is by itself immunosupressive in the host species (16).

Several technical questions will also persist. First, there is the question of purity of any preparation for human inoculation. Second, any design, however successful in the laboratory, has to take into account the eventual scale-up necessary for human immunization.

Results with Subunit Proteins. Initial profiles of HTLV-III/LAV gels from virus preparation at various stages of purity, and from several cell types, revealed that the "purified" virus was an extremely complex preparation comprised also of cellular proteins which sedimented as particulates in the virion density-sedimentation constant "window" (17). The selective accumulation of gp120 was particularly scarce in that several minor bands appeared in that area.

A powerful approach, combining a selection of amphopathic proteins into an immunostimulatory matrix, was attempted to obviate this problem. It was hoped that some major viral core proteins would also be eliminated in the process. Using quil A, matrices of approximately 35nm were formed by using whole HTLV-III/LAV by lysing intact virus through a gradient system. These matrices, termed immune stimulating complexes (ISCOMS), were readily formed with HTLV-III/LAV. The content of ISCOMS was examined electrophoretically and several prominent proteins were concentrated. Viral p17, gp41, and to a lesser degree gp120 were present; several unknown proteins of p32 and p38 size were also present in significant quantities. The major core protein p24 was effectively excluded from ISCOMS.

In sample preparation of ISCOMs formed from HTLV-IIIB virions (Fig. 1), structures of about 35 nm formed if sufficient amphopathic proteins were incorporated into the matrix. Little if any contaminating debris remained after purification (Fig. 2). ISCOMS induced antibodies in mice, rabbits, goats, horse, and rhesus monkeys after several immunizations in the range of 1–10 mg of total protein. Only in mice were untoward effects observed. The antibodies were induced against all of the proteins concentrated from ISCOMS content, as defined in RIP and Western

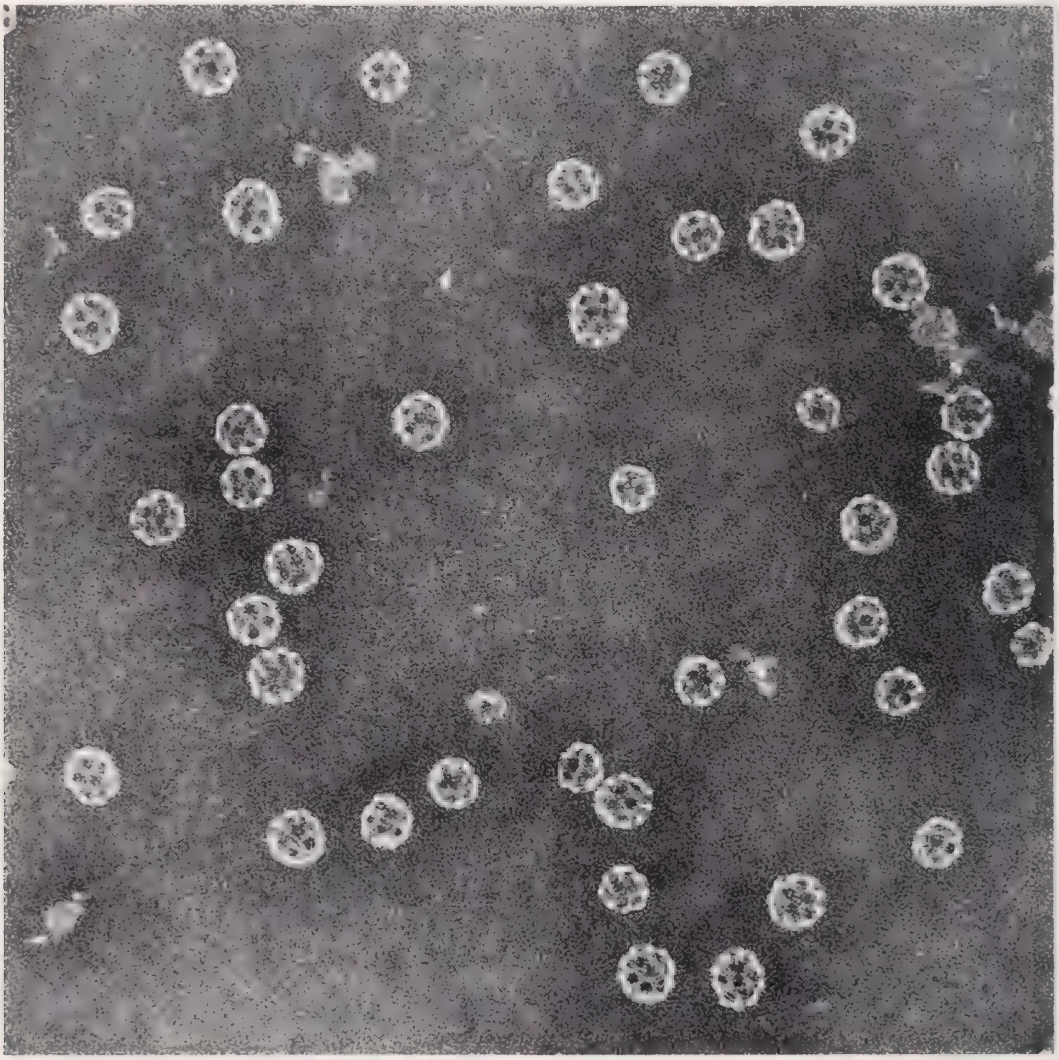

Fig. 1. Electron photomicrograph of immune stimulating complexes (ISCOMS) produced by lysis of HTLV-III virions. Proteins which have both hydrophilic and hydrophobic domains are incorporated into 35 nm matrices. ISCOMS do not form without such proteins, which constitute electron-dense elements of the structures. Orientation of highly immunogenic epitopes is thought to resemble natural configuration, and has been found to be a most effective way of eliciting strong humoral antibody response in several systems. *Figure produced by the NCI Contract, Program Resources Incorporated, at Frederick Cancer Research Facility.*

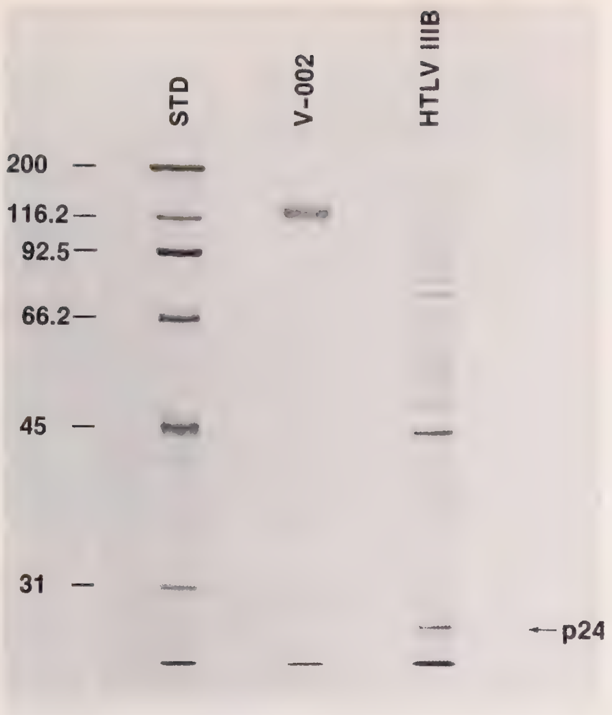

Fig. 2. Preparation of highly purified major viral glycoproteins of HTLV-III. Based on molecular weight standard (STD) markers, the gp is approximately 120,000 daltons in size. V-002, a standardized lot of an early vaccine preparation currently being tested in primates for immunogenicity. HTLV-IIIB, protein profile of purified virions of HTLV-III. Preparations are still very impure, although the major core protein, p24, is prominent; gp120 is barely detectable. *Figure produced by the NCI Contract, Program Resources Incorporated, at Frederick Cancer Research Facility.*

blot (WB) assays (17). The prominence of the p32 and p38 was so interesting that these were isolated and in part sequenced. When a search was performed for homology with known DNA sequences, the p32 and p38 clearly corresponded to the various DR chains derived from the H9 cell line (Henderson et al., unpublished data). When the antibodies were assessed in neutralization assays against the homologous HTLV-IIIB, only low levels of activity were observed, presumably because of the paucity of the gp120.

Purified gp120. A purification of gp120 was next attempted from virions, cell membranes, and post-virion supernates. A major tool was the formation of immunoabsorbent columns of pooled antibodies from virus-positive selected individuals whose reactivity was directed primarily against the *env* gene products.

Using various extraction modifications, pure gp120 was isolated in milligram amounts and purified to homogeneity by PAGE. The virus itself was a rather poor source of gp120. Evidently, more gp120 remained on the cell surface, or became detached from the virion so that it was found in the supernatant fraction after virion sedimentation. Regardless of source, the final purification was performed by elution from immune absorbent columns prepared with human antibodies strongly reactive against gp120. The pure gp120 did not form ISCOMS.

Upon inoculation into mice, rabbits, goats, horse, and rhesus monkeys, reactive antibodies were induced by as little as 10 μg of protein in two immunizations. Other viral proteins were not recognized by this antibody (18). Later, the gp120 was also deglycosylated by endo F, an effective endoglycosidase which removes most sugar linkages. The residual size was a p58 molecule which also induced antibody. In goats, Freund's adjuvant was used; and in the rhesus, the muramyl dipeptide, following previously effective protocols in another retroviral system.

In summary, neutralization data demonstrated clearly that in several tests, powerful neutralizing antibody was induced in all the species tested (19). Neutralization indices of 3–4, expressed as the log difference between titers in the presence of antibody and its absence, were observed; this value is considered a high titer for antibodies against retroviruses. Additional tests of neutralization, using inhibition of HTLV-III reverse transcriptase or the detection of p24 as parameters of viral replication, were used for confirmation. Neutralization was initially type-specific in that only homologous or very closely related virus was well neutralized. With later inoculations, some broadening of response was observed, so that a very different variant from HTLV-III, the RFII, was partially neutralized. It is hoped that as with mouse retroviruses, hyperimmunization could result in a predominance of group-specific neutralizing antibody (15). Deglycosylated p58 moiety of gp120 also induced neutralizing antibody (our unpublished data). This feature may be particularly important in the search for genetically engineered proteins and synthetic peptides.

When several of these antibodies were examined for a complement-mediated cytolytic effect on human cells infected with various HTLV-III variants, it was observed that a clear level of cytotoxic antibody existed. Tests were performed with both trypan blue exclusion and chromium release assays with appropriate complement and uninfected T4 cell controls. Antibody-dependent cytotoxicity was highly type-specific for the virus in question, and cells infected with even very closely related virus variants were not killed. Many human sera were tested in conjunction with the anti-gp120 animal sera, but no human serum, including those with high group-specific neutralizing antibody titer, ever showed any cytotoxicity. The latter phenomenon was a major difference between the experimental and natural anti-gp120 containing sera (P. Nara et al., unpublished data). The induction of complement-mediated cytolitic effect by experimental vaccine materials may be significant, especially if under natural conditions, infection occurs with intact cells.

Genetically Engineered and Synthetic Peptides of HTLV-III. The obvious direction is to make use of modern molecular technology to make large quantities of antigen in the absence of other viral material. As soon as DNA clones of HTLV-III became available, a number of groups expressed proteins in bacteria. Both the *gag* and the *env* regions were readily expressed in various sizes. The cleanest and most workable peptide was a segment produced from the 3′ half of the *env* gene and was designated as 121 (20). The peptide represented the 5′ end of the *tmp,* including the first hydrophobic region. The peptide reacted well with essentially all HTLV-III antibody-positive human sera tested, implying that the major reactive determinant of gp41, pathognomonic in confirmation tests, was present on the synthetic molecule. Peptide 121 was also inoculated into a number of species, including rhesus monkeys. Immunoreactive antibody was produced, but no neutralization has so far been observed.

Various 5′ to 3′ segments of the gp120 were also expressed in bacteria. Some of these were very large and spanned the gp120-gp41 cleavage junction. None were glycosylated, none entered ISCOMS, and all were extremely difficult to purify and to work with. We tested eight preparations, and only two of them induced antibodies reactive with gp120. One of these has recently been demonstrated to induce some type-specific neutralizing antibody. However, it is clear that yeast and mammalian cell technologies, using various inductions and superpromotion devices, will be able to make large and more nearly "native" products. Some of them apparently are in late developmental steps by a number of intramural and commercial concerns. Some success in the formation of a large complex, glycosylated gp130 sequence containing secreted, readout product from mammalian cells has been reported. This recombinant DNA vector had special viral promoters built into the DNA. The product induced antibodies reactive with gp120. At least some neutralization of homologous virus was observed (20a).

Synthesis of peptides representing contiguous regions of interest within a genome is now possible. Orientation and stabilization of putative epitopes and coupling technologies make this an attractive approach. One of these studies resulted in an 18-mer sequence, computer-selected for hydrophilicity and secondary structure prediction. The localization was on the HTLV-III *tmp* gp41 between positions 735-752. Upon immunization of rabbits, the resulting antiserum recognized the gp160 precursor *env* glycoprotein. Human sera which contained HTLV-III antibodies also reacted well with this peptide. Thus it was demonstrated that a totally synthetic peptide could contain epitopes found in native HTLV-III *tmp* (21).

A second group searched for peptides from the gp120 region which were conserved among divergent HTLV-III/LAV isolates and which contained predictably correct configurations. Several of these were made and assessed by the Duke University group (B. Haynes, personal communication). Antibodies reactive with native gp120 could be induced, and are currently being examined for neutralization. Although some recent data show that contiguous peptides could induce protective antibodies, other advanced computer-generated graphics show that for several viruses, all epitopes defined by monoclonal neutralizing antibodies were discontiguous, or contributed by chain folding of two peptides (22).

Infectious Recombinant Viruses Expressing HTLV-III/LAV Genes. Although current approaches use adenoviruses and herpes viruses as carriers, infectious recombinants based on vaccinia are by far the most advanced. Recently, two groups reported success in inserting the complete or partial *env* gene HTLV-III or LAV into vaccinia. The placement was downstream from the vaccinia transcriptional promoter and into the thymidine kinase (*tk*) gene. Residual *tk* sequences in the plasmid facilitated homologous recombination. Recombinants were isolated as *tk* variants by selective media (23, 24). Vaccinia vector mediated the expression of the HTLV-III/LAV *env* gene in a number of cell systems, not only in human cells. The synthesized proteins were comigrating with native gp160, gp120, and gp41 molecules of the virus, and appeared to be fully glycosylated. These proteins were not only immunoreactive with human sera, but also induced antibodies in mice and macaques which were specific for the corresponding *env* gene pro-

TABLE 3

Overview of Progress in Vaccine Research against AIDS

Antigens		Antibodies in lower species			Antibodies in rhesus			Higher primates
Preparation	Method	Reactive	Neutralizing	Cytotoxic	Reactive	Neutralizing	Cytotoxic	
Subunit composites ISCOMs	Amphopathic antigens in matrices	to p17 gp41 gp120 cell antigens	Low poor	NT	to p17 gp41 gp120	Low – poor	NT	—
Purified gp120 from virions or infected cells, native/ deglycosylated	Native, PAGE with adjuvants Freund's or MDP	to gp120 only	strong, type-specific	Highly type specific	to gp120	Good, type	Negative	Under test
Genetically engineered from bacteria from cell cultures	gp41 (#121)	to gp41, gp160	negative	NT	to gp41	NT	NT	—
	gp120 pieces (8 assorted)	some induced anti gp120	most negative	NT	NT	NT	NT	
	gp120 composite	anti gp120	one positive low level	NT				Under test
Synthetic peptide (with carrier)	from gp41 conserved sequences of gp120	to gp41, gp160 to gp120	NT	NT				Under test
Infectious recombinant virus	Vaccinia vector *env* gene	with gp41 with gp120	NT	NT	Reactive (preliminary)			Under test

ISCOMS, immune stimulating complexes; MDP, muramyl-dipeptides; PAGE, polyacrylamide gel electrophoresis; NT, not tested.

teins of the virus. Whether these antibodies are neutralizing has not been demonstrated as yet. However, analogous recombinant vaccinia variants with other retroviral *env* genes could stimulate both humoral and cell-mediated immunity, and actually protect animals against the challenge of a murine leukemia virus (25). This approach may be very rapid; however, questions of possible multiple sequential inoculations of vaccinias containing other, less related HTLV-III *env*'s has to be considered, especially in the light of individuals previously immunized with vaccinia.

Animal Models for Assessing Protection. Rhesus monkeys have been experimentally infected with and have produced antibodies to HTLV-III. However, the frequency of seroconversion and the occasional temporary release of virus are inadequate. Chimpanzees, in contrast, have been readily infected with HTLV-III/LAV, make essentially the same profile of antibodies as do humans, and shed virus from that time onward (26). The virus does cause CPE in chimpanzee T4 cells. Occasional lymphadenopathy and temporary T4 depression were observed, but AIDS has never been seen in this species up to this time.

Thus the chimpanzee presents a ready model to determine whether primary infection with HTLV-III/LAV can be prevented in a vaccinated animal. If good neutralizing antibody could also be induced safely in this species, a challenge with titrated homologous virus could take place. Antibodies could be induced and tested with less-related HTLV-III variants as well, to determine whether cross-neutralization occurs in vitro prior to in vivo challenge, so as not to waste this scarce resource. Multiple routes of inoculation will also have to be considered. Because of the small number of available animals, very conservative approaches will take precedence. Initial inoculations of purified gp120 into chimpanzees have been initiated.

To determine what preparation could actually protect from the disease itself, the simian T-cell lymphotropic virus III (STLV-III) model may be very useful (27). A close relative of HTLV-III has been shown to cause an AIDS-like syndrome in several species of macaques. The STLV-III has been isolated, in part characterized, and appears to be closely analogous to HTLV-III/LAV. Based on current technology, isolation of the gp120 of the pathogenic macaque virus is feasible in any one of the formats cited above. Thus, parallel experiments of induction of antibody and subsequent challenge could take place in this monkey model. This time between virus inoculation and disease has generally been less than six months.

Overview and Directions

The unavoidable questions of timing of movement on several fronts, the question of relative priority of one approach over the other, and the question of practical scale-up impinge on the decision process. A broad plan has to take cognizance of directional decisions which favor one over another approach. Successes or failures may not be qualitatively measurable in early stages. A plan for the future must be flexible as targets of opportunity arise. The pace of research in AIDS has shown that one has to make room for maneuverability. The private sector is beginning to make a significant contribution, especially in the area of recombinant DNA technology. Such private efforts must be integrated into the federal and university efforts and for their participation should be specifically facilitated. The question of liability for producers of AIDS vaccines will be a major stumbling block unless appropriate legislative measures are implemented.

A simplified overview of progress so far in experimental and developmental vaccine research against AIDS is presented in Table 3. The rate of progress may be variable, as the blank spaces in the table indicate; however, the experimental challenge system now initiated in the chimpanzee will be a key point to consider. The number of currently available preparations with assorted virus variants, adjuvants, protocols, and challenge systems exceeds the number of available animals. Currently, only animals previously exposed to all human hepatitis viruses are being considered. Great care has to be taken to use this scarce and valuable resource judiciously. The initial experiments have been started in this species. Based on the material summarized here, cautious optimism may be in order. On the other hand, the complexity of this virus system is probably greater than most other human viral diseases.

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Antiviral Chemotherapies Directed against HTLV-III/LAV

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AIDS is a disease characterized by extensive immunosuppression that predisposes patients to life-threatening opportunistic infections and malignancies (1). To date, over 20,000 cases of AIDS have been reported in the United States; the incidence and prevalence of this disease continue to increase. No person with AIDS has recovered, and the vast majority have died within 3–4 years.

Human T-lymphotropic virus type III (HTLV-III)/lymphadenopathy-associated virus (LAV) is a newly discovered retrovirus etiologically linked to AIDS and AIDS-related disorders (2–6). HTLV-III/LAV preferentially infects and destroys helper/inducer (OKT4+/Leu3+) T-lymphocytes in vitro (7). HTLV-III/LAV is generally spread by intimate sexual contact or by the administration of blood products, and occasionally by the maternal-fetal route (8). Many patients who develop HTLV-III/LAV infection are asymptomatic when they transmit the virus to contacts, because a long latency interval (months to years) may exist between infection and clinical manifestations of illness.

Prior to the identification of the etiologic agent, attempts at therapy focused on treatment of the multiple opportunistic infections or neoplasms, particularly Kaposi's sarcoma, or on enhancement of immune function by, for example, interferons, interleukin-2, or bone-marrow transplantation (9–11). Despite occasional temporary clinical improvement in certain patients, the overall prognosis has remained devastating.

The discovery of HTLV-III/LAV and its identification as the etiologic agent causing AIDS and AIDS-related disorders in 1983–1984 initiated consideration of therapies specifically directed against the underlying agent. Detectable viral replication in vivo characterizes the HTLV-III/LAV infection in patients with AIDS and AIDS-related disorders, at least early in the disease. Active replication of HTLV-III/LAV, with the resulting depletion of helper/inducer (OKT4+/Leu3+) T-lymphocytes, is believed to be important in initiation and progression of AIDS (2). Thus, antiviral therapy which specifically inhibits HTLV-III/LAV replication may be of potential benefit in patients with AIDS and AIDS-related disorders. In addition, such therapy may prevent the development of AIDS or AIDS-related disorders in asymptomatic patients infected with HTLV-III/LAV and may reduce the transmissibility of the infectious virus from person to person.

The rationale for anti-HTLV-III/LAV chemotherapy has changed in recent years. The reason for a change in attitudes among virologists is complex, but is related to advances in the understanding of the molecular biology of viral replication (12). As molecular biologic techniques have improved, it has become possible to provide intervention strategies predicated on molecular mechanisms. A second factor in the change of emphasis by virologists is the realization of the clinical impact of persistent infections, either as reservoirs of infection for transmission or for clinical disease. Herpes viruses are characterized by recrudescence and HTLV-I infections are characterized by a proliferative outcome, adult T-cell leukemia (13). In AIDS, the chronicity of HTLV-III/LAV infection and its inexorable downhill course alter the usual preventive focus of disease management. Although immunoprophylaxis and

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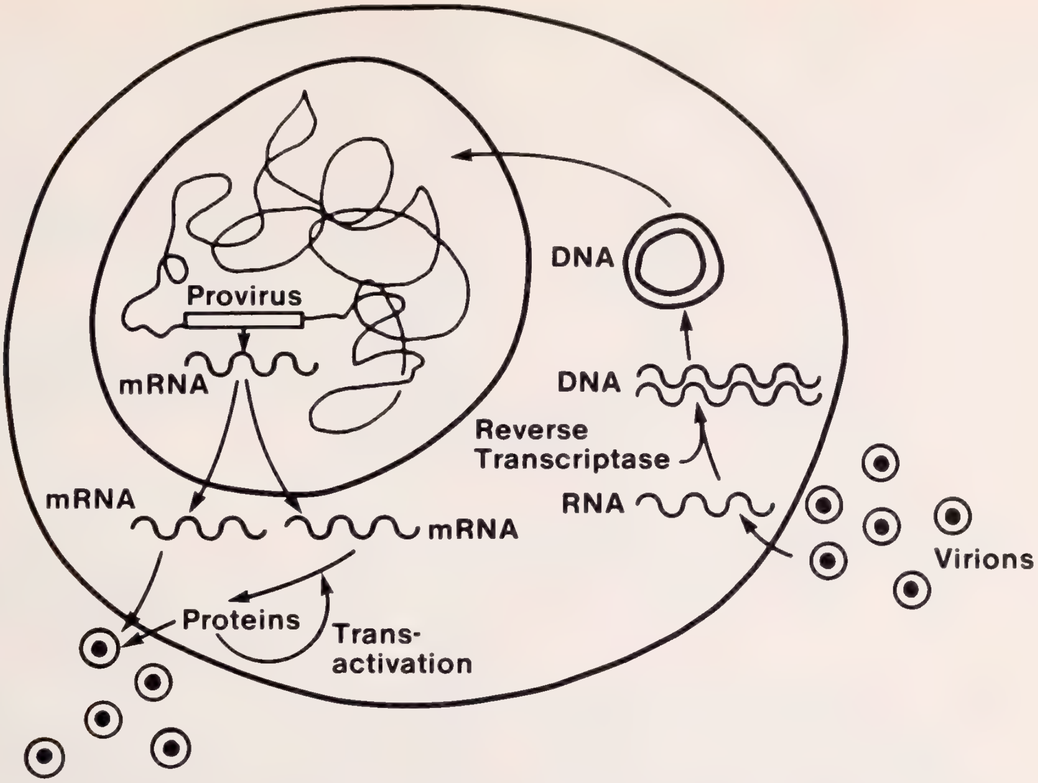

Fig. Life cycle of HTLV-III/LAV. Retroviral replication cycles begin when virus particles are absorbed to specific cell receptors. After penetration, virus is uncoated in cytoplasm and viral RNA is transcribed by reverse transcriptase, which uses a host transfer-RNA primer to initiate reaction and proceeds through an RNA-DNA hybrid intermediate. Single-stranded complementary DNA is made initially, then synthesized into linear, double-stranded proviral DNA. Proviral DNA then circularizes; some is integrated into host chromosomal DNA. Provirus is subsequently expressed by transcription of integrated DNA sequences into viral messenger RNA, which, during post-transcriptional processing, is modified at both ends. Virus-specific mRNA is translated into viral proteins, some produced after cleavage of viral precursor proteins. A trans-activating protein provides the virus with a positive feedback loop acting to increase production of itself and other viral proteins post-transcriptionally. Mature HTLV-III/LAV particles have outer envelopes containing viral glycoproteins synthesized and glycosylated in rough endoplasmic reticulum and transported through Golgi apparatus to plasma membrane. Virus particles are assembled and released by budding through plasma membrane in areas where viral glycoproteins congregate.

interruption of transmission are important elements of any comprehensive control plan for HTLV-III/LAV infection, so too is treatment. Potentially, as treatment improves, there may even be a role for chemoprophylaxis.

HTLV-III/LAV, as compared to a number of viruses of medical importance such as hepatitis B, is not highly infectious (14). Thus, it is a reasonable early goal of anti-HTLV-III/LAV chemotherapy to prevent transmission of HTLV-III/LAV to sexual contacts of infected individuals. Such a use of chemotherapy would be partially analogous to the use of drugs for chemoprophylaxis of *Mycobacterium tuberculosis*. In HTLV-III/LAV infection, there is a restricted level of viral replication in that only a minority of infected cells express virus at any one time (15). Therefore, it is possible that transmission could be interrupted by as little as a consistent 50%–90% reduction in virus production in infected individuals.

Historically, efforts to discover effective antiviral agents have met with limited success. Since the viral and host replicative mechanisms were considered to be similar, it was difficult to envisage inhibition of viral functions without unacceptable toxicity to the normal host cells. Recently, it has become clear that viruses, as they infect host cells, carry or produce unique enzymes (herpes-induced thymidine kinase and DNA polymerase), which can be inhibited to achieve therapeutic specificity (16). Human retroviruses such as HTLV-III/LAV provide additional targets for the development of specific chemotherapeutic agents.

Effective anti-HTLV-III/LAV chemotherapy will involve two basic elements. One approach is to interfere with cell-to-cell transmission of HTLV-III/LAV. The second approach is to restore the patient's immune competence after damage by the destruction and dysfunction of lymphocytes following virus infection; a discussion of this approach is beyond the scope of this review, which focuses primarily on antiviral efforts.

Interfering with Cell-to-Cell Transmission

Based on our knowledge of the replicative cycle of animal and human retroviruses (Fig.), there are two ways to view approaches to retrovirus chemotherapy (17): via the infecting cell, or via the susceptible cell.

In the Infecting Cell. First, in what is referred to as the "infecting" cell, attempts could be focused on interference either with transcription of the integrated provirus or with viral-specific functions that occur before the virus leaves the infected cell.

There are no established methods for attacking the integrated proviral copy. Efforts that would focus on transcription of the integrated provirus must consider issues of altering the transcription of the cellular DNA-dependent RNA polymerase, *pol II*.

The HTLV-III/LAV genome (*tat-III*) encodes a unique trans-activating protein, which apparently acts post-transcriptionally to increase the rate of viral protein production from viral messenger RNA (18, 19). Since the trans-activating protein is required for viral replication, it offers a potential target for selective inhibition. A short segment of complementary DNA, the "antisense" oligodeoxyribonucleotide, can bind to a segment of the HTLV-III/LAV messenger RNA and potentially block the binding of the trans-activating protein or directly block ribosomally mediated translation (20). Attempts to inhibit the *tat-III* gene product with the use of an "antisense" oligodeoxyribonucleotide, as an example, are reasonable, but raise questions regarding the specificity of such processes (21). Similarly, other attempts to interfere with assembly of viruses at the plasma membrane are likely to be targeted to a unique viral function. However, in each of these situations, these strategies cannot be considered ready for practical application.

In Susceptible Cells. A second approach to retrovirus chemotherapy is to interfere with replication of the infecting virus in "susceptible" cells. Destruction of the OKT4 molecule, the putative HTLV-III/LAV receptor, might block viral attachment (7, 22, 23). However, such a plan raises many questions about the normal function of the OKT4 molecule and leaves open to question what other immune effects might follow. But since the likely viral component that binds the OKT4 molecule is the gp120 envelope-gene product, it is possible that this approach may at some point be feasible (24). In fact, as an increased understanding of the envelope-gene product evolves, it may not be entirely unreasonable to consider interference with OKT4 binding.

Viropexis—the cellular uptake of virus—and the uncoating of the viral nucleoid within viral phagosomes are areas that are distinct from cellular processes, but raise major questions concerning feasibility.

Focus: Reverse Transcriptase

The exposure of the viral RNA from the uncoated virus with its essential catalytic component, the RNA-dependent DNA polymerase, has been the primary focus of antiviral attack. The replicative cycle of retroviruses may be particularly vulnerable to chemotherapeutic attack because reverse-transcriptase-catalyzed transcription of genomic RNA into complementary DNA is unique to the life cycle of retroviruses and is not known to occur in normal eukaryotic cells (25, 26). The virus-induced enzymes often differ from their normal cellular counterparts in broadened substrate specificity, cofactor requirements, and other properties (16). The differences are exploitable and may impart the needed specificity to inhibit virus replication without concomitant unacceptable toxicity to mammalian cells. Moreover, reverse transcriptase is a multifunctional enzyme and, as such, offers attractive sites for the development of chemotherapeutic agents to inhibit retroviruses.

Properties of Reverse Transcriptase. HTLV-III/LAV reverse transcriptase (RNA-dependent DNA polymerase) has unique properties compared to normal-cell polymerases. The following steps result from the direct action of the HTLV-III/LAV polymerase gene product and are the most susceptible to chemotherapeutic intervention (27):

1. Synthesis of an RNA-DNA hybrid using the host lysyl transfer-RNA as a primer (28).
2. An RNAse-H activity that hydrolyzes the RNA from an RNA-DNA hybrid, creating a single-stranded DNA molecule. There are no error-reading or proofreading functions for this enzyme.
3. The single-stranded DNA molecule is the template for the DNA-dependent DNA polymer-

TABLE 1
Status of Chemotherapeutic Agents with Potential Anti-HTLV-III/LAV Activity

Drug	Proposed mechanism of action	Clinical trial results
Suramin (36,40)	RT inhibitor	Virustatic, no clinical or immunologic improvement
HPA-23 (antimoniotungstate) (37)	RT inhibitor	Virustatic, no clinical or immunologic improvement
3′-Azidodeoxythymidine (AZT) (44)	Chain termination, RT inhibitor	Virustatic, short-term clinical and immunologic improvement
Ribavirin (47)	5′-capping of viral mRNA, ↓ GTP pools	Being tested
Phosphonoformate (Foscarnet) (49)	RT inhibitor	Being tested
Alpha-interferon (52)	Viral budding?	Partial activity against Kaposi's sarcoma
Rifabutine (Ansamycin) (63)	RT inhibitor?	Being tested
Al-721 (64)	Altered viral envelope or receptor	Being tested
Imuthiol (dithiocarb) (65)	Unknown	Being tested
Isoprinosine (inosine pranobex) (65)	Unknown	Being tested
2′,3′-Dideoxynucleosides (53)	Chain termination, RT inhibitor	Not tested
Oligodeoxyribonucleotides ("anti-sense" DNA) (21)	Binding to viral genetic sequences	Not tested

RT, reverse transcriptase.

ase activity of the reverse transcriptase. The action of this enzyme generates the linear double-stranded copy of the viral information. This linear copy is transported to the nucleus, and circularization of the DNA follows.

4. By utilizing the product of the integrase domain of the *pol* gene, which produces single-stranded cuts and superhelical DNA and has an unwinding topoisomerase activity, the viral DNA becomes integrated. Integration and productive infection requires at least one round of cellular DNA replication followed by mitosis. From the integrated provirus, transcription can begin again using the cellular DNA-dependent RNA polymerase, the *pol-II* enzyme.

Inhibition of Reverse Transcriptase. Prior to 1970, retroviruses were known to be a frequent cause of naturally occurring tumors in animals. In 1970, reverse-transcriptase was discovered by Temin and Baltimore (25, 26). In the hope of discovering new antitumor agents, efforts were directed to identifying reverse-transcriptase inhibitors in animal systems. Approaches to inhibition of the reverse transcriptase enzyme that are theoretically possible include (29):

1. Compounds that bind directly to the enzyme. These are relatively large molecules, the prototype inhibitors being suramin and the rifampycins (30). These inhibitors are tested by screening for polymerase inhibition in vitro, followed by correlation with antiviral activity in cell culture experiments.

2. Template/primer analogues. Modified polynucleotides such as thiolated polycytidylates compete with the normal template for the template-binding site of the enzyme (31).

3. Substrate analogues. Substrate analogues, such as 3′-azidodeoxythymidine, should be effective inhibitors because of the infidelity of the retrovirus reverse transcriptase. Analogues with mechanisms of action other than chain termination may be specific and potent inhibitors with minimal associated toxicity.

4. Template/primer binding compounds. Actinomycin-D and ethidium bromide bind to specific base pairs of the template (32). Oligodeoxyribonucleotides also bind to single-stranded nucleic acids (21).

5. A category of compounds with other modes of action, such as interference with the integrase or the RNAse-H activity. Also included would be drugs such as ribavirin that have multiple modes of action, one of which may directly or indirectly affect polymerase function.

Agents under Study

The majority of the currently utilized clinical agents directed against HTLV-III/LAV have some anti-reverse-transcriptase activity, suggesting that the retrovirus target with maximum selectivity and associated minimal toxicity is likely to be the viral polymerase gene product. Toxicity is already a major clinical issue with the compounds being tested, probably due to a lack of adequate selectivity.

Chemotherapy for retroviruses differs from chemotherapy for other viruses in a single key respect. Retroviruses replicate maximally in cells with maximal DNA synthesis, particularly in cells in logarithmic growth phase (33). There is no evidence that retroviruses will replicate in cells without DNA synthesis. Therefore, it is particularly important that any drug effect on cellular DNA metabolism be explored before conclusions on viral inhibition are made. Correlation of viral and cellular effect will need to be determined.

In vitro models to screen biological or pharmaceutical agents with potential activity against infectious HTLV-III/LAV are the initial testing ground for agents prior to consideration for clinical trials (34). A number of antiviral agents have exhibited efficacy and have been reported to inhibit the infection by HTLV-III/LAV of established T-cell lines or cultured T-cells in vitro (35) (Table). All effective agents studied to date have a dose-dependent inhibitory effect on HTLV-III/LAV infectivity in vitro. Suramin, HPA-23, and 3′-azidodeoxythymidine, inhibitors of retroviral reverse transcriptase, have been reported to reduce the number of HTLV-III/LAV infected cells in vivo (36–38).

Specific Compounds

Suramin Sodium. A hexasodium derivative of naphthalene-trisulfonic acid, suramin sodium has been used as an effective agent for the treatment of African onchocerciasis and trypanosomiasis. It has been shown to be a potent inhibitor of the purified reverse transcriptase of HTLV-III/LAV and other retroviruses (39). Suramin protected cultured T-cells against HTLV-III/LAV infectivity and cytopathic effects (40, 41).

Based on these results, clinical trials of intravenously administered suramin were initiated in patients with AIDS. A six-week treatment regimen proved efficient in temporarily suppressing virus replication, but despite an in vivo virustatic effect, no clinical or immunologic improvement was observed (36, 42). Renal failure, adrenal failure, fever, photosensitivity, burning sensation of the skin, and skin eruptions were clinical toxicities associated with it. All side effects were reversible on discontinuation of the drug. Clinical trials have been halted due to unacceptable toxicity. The preliminary results with suramin therapy suggest that it does not have a role as single-agent therapy in HTLV-III/LAV disease.

HPA-23 or Antimoniotungstate. A mineral-condensed polyanion of ammonium 21-tungsto-9-antimoniate, HPA-23 acts as a competitive inhibitor of animal and HTLV-III/LAV reverse transcriptases (43). Its pharmacologic half-life is short (less than one hour) and it must be administered intravenously. As in the case of suramin, clinical trials revealed transient virustatic effects, but no evidence of clinical or immunologic improvement. Thrombocytopenia and elevated liver function studies were the major toxicities (37).

3′-Azidodeoxythymidine. A substrate analogue, 3′-azidodeoxythymidine (AZT) is converted to a triphosphate form by cellular enzymes, and this triphosphate form is utilized by HTLV-III/LAV reverse transcriptase (44). The 3′-substitution makes subsequent 5′ to 3′-phosphodiester linkages impossible (chain terminator of DNA synthesis). Cellular DNA polymerases are less susceptible to inhibition than reverse transcriptase.

Based on its potent inhibitory effects on HTLV-III/LAV in vitro, clinical trials of 3′-azidodeoxythymidine in patients with AIDS or AIDS-related complex were initiated. Only the highest-dosage regimens produced virustatic results. However, partial clinical and immunologic improvement occurred, particularly in those participants in the lower-dosage regimens (38). Randomized, double-blind, placebo-controlled studies of orally administered 3′-azidodeoxythymidine have been halted as a result of encouraging preliminary observations. Clinical trials have resumed in an open-label format adopted for ethical reasons. The accumulation of the agent within cells causes substantial depression of thymidine triphosphate, resulting in bone-marrow suppression and megaloblastic anemia.

Ribavirin. A guanosine analogue, ribavirin has broad antiviral activity against RNA and DNA viruses in vitro and in vivo (45). One proposed mechanism of action is the interference of the guanylation step required for 5′-capping of viral mRNA (46). In vitro, HTLV-III/LAV replication was only partially inhibited at relatively high concentrations usually associated with toxicity (100 μg/ml) in vivo (47). Clinical trials are in progress. No preliminary effects have been noted in the treatment of HTLV-III/LAV infection of infants (48). The major toxicities of this orally administered agent are anemia and liver enzyme elevations.

Phosphonoformate. The sodium salt of phosphonoformate, a pyrophosphate analogue, inhibits the reverse transcriptase of HTLV-III/LAV and various animal retroviruses, including visna virus, which is related to HTLV-III/LAV (49, 50). It is not readily usable as an oral agent. Accumulation of the drug in bone and acute renal failure are toxicities associated with it.

Interferons. Interferons are natural proteins produced during viral infection or after antigenic challenge that have broad-spectrum activity against viruses. All interferons appear to act indirectly by binding to specific cell surface receptors and inducing the production of cellular enzymes that have antiviral activity. Animal retroviruses and HTLV-III/LAV are reported to be sensitive to the antiviral activities of interferons in vitro (51, 52). The attack site in animals is the assembly and subsequent release of viral particles, although the molecular mechanisms underlying this inhibition are unknown.

Clinical trials were initiated in patients with Kaposi's sarcoma because of the antitumor properties of alpha-interferon. It is unknown if alpha-interferon is virustatic, but, despite no immunologic improvement, clinical remission of Kaposi's sarcoma occurred in a small percentage of patients (9). Toxicity involving the bone marrow (reversible suppression), central nervous system, and liver, along with a flulike syndrome, occur.

2′,3′-Dideoxynucleosides. 2′,3′-Dideoxyguanosine, 2′,3′-dideoxyadenosine, and 2′,3′-dideoxycytidine have recently been shown to inhibit the replication of HTLV-III/LAV and impart protection against the virus to T-lymphocytes (53). The mechanisms by which these analogues suppress the replication of HTLV-III/LAV is unknown. However, in purified DNA polymerase studies, 2′,3′-dideoxynucleoside-5′-triphosphates have demonstrated the capacity to bind to the triphosphate sites as well as act as substrates for addition to the DNA chain (54, 55, 56). Once added to the DNA strand, these molecules, being devoid of the 3′-hydroxyl group, can block chain growth by preventing the attachment of additional nucleotide residues. Thus, the biosynthesis of DNA is inhibited at two sites: DNA polymerase and chain termination (57).

In vivo, these derivatives may be anabolized by nucleoside and nucleotide kinases to their respective 2′,3′-dideoxynucleoside-5′-triphosphates, which inhibit DNA polymerases and incorporate into the 3′-position of the primer, resulting in premature chain termination. Thus, it is postulated that the 5′-triphosphates of the active nucleosides may be inhibitory to HTLV-III/LAV reverse transcriptase, and the favorable inhibitory kinetic parameters may impart a measure of selectivity. 2′,3′-dideoxycytidine has been selected for future testing in humans and is currently undergoing animal toxicity studies.

Nucleoside analogues which either compete with or retard the formation of the intracellular pool sizes of dGTP appear to be effective inhibitors of HTLV-III/LAV replication. Ribavirin causes an increase in TTP pool size and a decrease in dGTP pool size (58), whereas 2′,3′-dideoxyguanosine and 2′,3′-dideoxycytidine compete with the utilization of 2′-deoxyguanosine via salvage pathways.

In Vitro and In Vivo Comparison

Studies of suramin, 3′-azidodeoxythymidine, and 2′,3′-dideoxynucleosides have been sequentially investigated by Broder and his colleagues at the National Cancer Institute (40, 44, 53). The first two drugs have already reached Phase II clinical trials, so there is considerable data for comparison of in vivo and in vitro studies. Careful analysis of in vitro data reveals that none of the drugs studied to date is markedly specific. The level of drug that will inhibit 90% to 100% of the virus production is inhibitory in nearly all cytotoxic assays within a tenfold range of the 90% effective antiviral concentration. For example, Mitsuya et al found a dose-dependent decline in 3H-uridine uptake at levels twofold higher than that of the 3′-azidodeoxythymidine dose required for 90% viral inhibition (44). Immunologic functions of T and B lymphocytes were not totally inhibited with 20-to-100-fold higher doses of 3′-azidodeoxythymidine in short-term experiments. Clinically, 3′-azidodeoxythymidine has been found to have toxicity that limits drug dose.

In vitro, the removal of suramin or 3′-azidodeoxythymidine after infection allowed the appearance of virus production (41, personal observation). In the clinical trials, the virustatic effects of these agents have been temporary, depending on the drug levels, validating the in vitro findings.

In summary, the low therapeutic indices for suramin and 3′-azidodeoxythymidine and their transient virustatic effects using short-term toxicity and virus inhibition assays are partially confirmed by in vivo studies. These findings strongly support our need for new drugs and more vigorous validation of in vitro testing. Similar approaches have also been shown to validate anti-herpes-virus testing in vitro (59).

Drug Resistance and Combination Therapy

Drug resistance has been a major problem in treating bacterial infections with antibiotics. In general, the more specific a given drug, the more likely that drug-resistant viral mutants will develop. Similarly, a drug with only a single mode of action will tend to be less toxic, but also more apt to promote the development of resistance. The ability of the HTLV-III/LAV provirus to exist in a latent form in the host cell genome suggests that prolonged treatment is likely to prove essential. Thus, combination therapy with antiviral agents may afford the advantage of discouraging the selection of drug-resistant mutants. Also, the use of multiple effective antiviral agents with various mechanisms of action, at doses that minimize side effects, may synergistically lead to chronic suppression of viral replication, thus decreasing the spread of virus and halting the progression of disease (41).

Summary

The growing AIDS epidemic has given a strong impetus to rapid identification of antiviral agents which could immediately be brought to clinical trial. Based on our knowledge of the replicative cycle of animal and human retroviruses, most current research devoted to the development of specific anti-HTLV-III/LAV therapy has focused on the inhibition of reverse transcriptase, an enzyme unique to retroviruses, not found in normal eukaryotic cells (25).

Suramin and HPA-23, inhibitors of the reverse transcriptase of animal retroviruses, were the first antiviral agents tested for inhibitory activity against HTLV-III/LAV in vitro (37, 40). Their reported efficacy rapidly led to clinical trials in patients with AIDS and AIDS-related disorders (36, 37). Preliminary clinical studies revealed that suramin and HPA-23 could inhibit HTLV-III/LAV replication in vivo, but no clinical improvement occurred. Antiviral agents alone, while halting viral spread, may not in and of themselves be curative. The use of immune modulators may be needed as adjunctive therapy to restore the HTLV-III/LAV-damaged immune system.

Because the variable natural history of HTLV-III/LAV infection renders anecdotal reports almost meaningless, carefully controlled clinical trials, randomized and double-blind, will need to be performed. Although a number of antiviral agents are now being considered for experimental therapy of HTLV-III/LAV infections, to date no therapy has been shown to cure HTLV-III/LAV infection or restore the underlying immunodeficiency. Moreover, the chronicity of infection and the propensity of the virus to infect the neurologic system require that we explore new classes of drugs which have the potential for oral administration and penetration across the blood-brain barrier (60–62).

In the short time since the discovery of the etiologic retrovirus of AIDS, significant progress has been made toward identifying inhibitory agents. However, currently available anti-HTLV-III/LAV agents are virustatic and show activity near their toxic levels, thus limiting their usefulness and reinforcing the need to discover new and better compounds. Innovative strategies targeted to pharmacologic intervention against HTLV-III/LAV through viral research in conjunction with drug development may ultimately yield effective treatment of patients infected with HTLV-III/LAV.

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Diagnostic and Prognostic Factors in AIDS

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Abstract

Clinical evaluation of patients with AIDS, AIDS-related complex, or asymptomatic infection with human immunodeficiency virus (HIV) includes immunology laboratory detection of antibodies to HIV and measurement of immune impairment, especially of those factors relating to prognosis. In this report we outline the use of and differences between individual enzyme-linked immunosorbent assays for HIV antibodies for diagnosis. We stress the need for both quantitative and qualitative assessment of HIV antibodies, and emphasize the importance of serial testing of individuals, in addition to in-depth characterization of single serum samples, whenever the person is at risk for developing HIV infection. We review the quantitative assessment of CD4 (T4 helper/inducer) T-cell numbers and CD4:CD8 (T4:T8) ratio for extent of decrease, which correlates strongly with poor prognosis. We note that stimulation of the immune system by HIV infection may be responsible for increases in OKT10 antigen expression on CD8 T cells; such an increase is an index of poor prognosis in patients with AIDS-related Kaposi's sarcoma and possibly in other groups with HIV infection.

Acquired immune deficiency syndrome (AIDS), the severe disease state resulting from human immunodeficiency virus (HIV) infection, and the less severe forms of the disease, often termed AIDS-related complex (ARC), continue to increase rapidly. It is likely that more than one million Americans are infected with the HIV (HTLV-III/LAV) virus that causes AIDS. Furthermore, many persons who may have been exposed to HIV by blood transfusion, sexual intercourse, or other means need to know if they have contracted HIV infection, as do their physicians. All who have the infection wish to know the prognosis. This widespread concern is bringing to all physicians a need to understand the nature and status of the tests which establish the diagnosis of HIV infection and the tests which relate to prognosis, that is, the likelihood of acquiring AIDS for those who are seropositive, and the likelihood of survival in those who have the diagnosis of AIDS.

The clinical features which establish the diagnosis of AIDS and ARC and which relate to prognosis have been reviewed (1–3). In this report, we focus on the immunologic tests needed to establish HIV infection, and those needed to evaluate the extent of immune deficiency—the central factor in disease pathogenesis. Evidence is presented indicating that prognosis degenerates with reduction in CD4 T-cell number and CD4:CD8 (T4:T8) ratio and with increased expression of OKT10 antigens.

Immunologic Diagnosis of HIV Infection

Presence of antibody to HIV antigens is the primary means of establishing that persons have been or are carrying the virus. Antibodies to HIV

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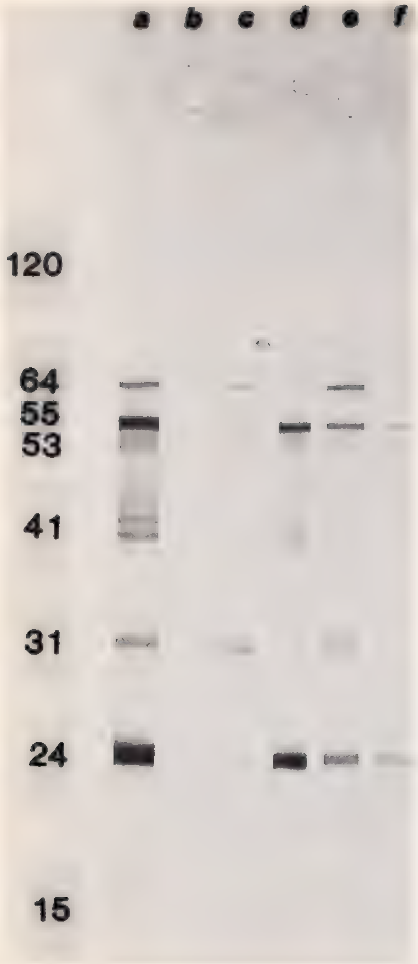

Fig. 1. Representative Western blot analyses of serum from persons with HIV infection, showing antibodies to the 8 major and other antigens of the virus; individual antigens indicated by approximate molecular weight. Lane a, typical strong positive; lane b, negative; lane c, weakly positive, all bands present; lanes d and e, atypical positive bands; lane f, positive—a recent seroconverter showing only antibodies to p24, p55.

are reported to appear as early as four to six weeks after infection with the virus. Antibodies are usually detected by serologic tests based on enzyme-linked immunosorbent assay (ELISA) methodology.

Eight Major Antigens. The HIV virus has 8 major antigens to which antibodies develop after infection (4, 5). These include antibodies to the gag gene products which are identified as p15 (p13 and p18), p24, and p55. A second major set of antigens are those for env (envelope) gene product; these are gp41, gp110, and gp160. Other antigens include reverse transcriptase (p66/p51) and endonuclease polymerase (p31).

Western Blot Confirmation. The Western blot (WB) technique is generally used as a confirmatory test to detect the presence of antibody to the HIV antigens. This test is done by reacting the suspect serum with HIV antigen, which is separated on SDS polyacrylamide gel, and transferred onto nitrocellulose membrane. Individual antibodies are delineated on these nitrocellulose strips. In positive Western blot tests, a variety of patterns are seen (Fig. 1).

ELISA False Positives. ELISA plates contain disrupted and immobilized HIV antigens which bind specific antibodies. Some HIV antigen preparations also contain HLA-DR antigens from the cell lines in which the HIV virus is grown (6). As a consequence, tests of sera containing antibodies to these HLA-DR antigens may give false positive results because of high levels of anti-DR antibodies in non-HIV sera. Some HIV preparations are grown in cells without HLA-DR antigens, and kits using such preparations avoid this complication.

ELISA False Negatives. Initial antibody responses are usually manifested by antibodies to p24 and gp41 antigens of HIV. In many patients one of these will appear exclusively before the other. Clearly there is a need for all ELISA tests to detect both of these antibodies. Unfortunately, some of the test kits that have been used fail to detect low levels of p24 (7) when this is the only antibody present early in immune response to HIV infection. ELISA test kits for HIV antibodies are available, however, which do detect these antigens (6, 8).

Quantity of Antibody and Seroconversion Timing. Another factor relevant to the diagnostic application of ELISA tests for HIV antibody is the quantity of antibody present. In practice, a certain optical density (O.D.) or equivalent measurement is designated as the threshold or cut-off point, which varies in commercial test systems (Fig. 2). In a study (6) of antibody levels as reflected in quantitative measurement by two different commercial test kits applied to the same sample population, 350 homosexually active men at risk for AIDS, approximately half the population demonstrated HIV seropositivity. In system A quite a number of samples are near the arbitrary cut-off point, a problem much less evident in test system B, which provides a more precise and reliable result (Fig. 2). In a recent study we determined that 25% of negative samples (9 of 36) in the upper 5th percentile of negative optical density went on to convert within 6 months by both ELISA and WB tests. It seems possible that at the time of the initial test they were beginning to develop antibody but had not yet reached the cut-off point. Their state of infectivity when seronegative remains to be established.

A group of 51 persons who seroconverted while under serial observation showed higher "negative" quantitative ELISA values. The mean

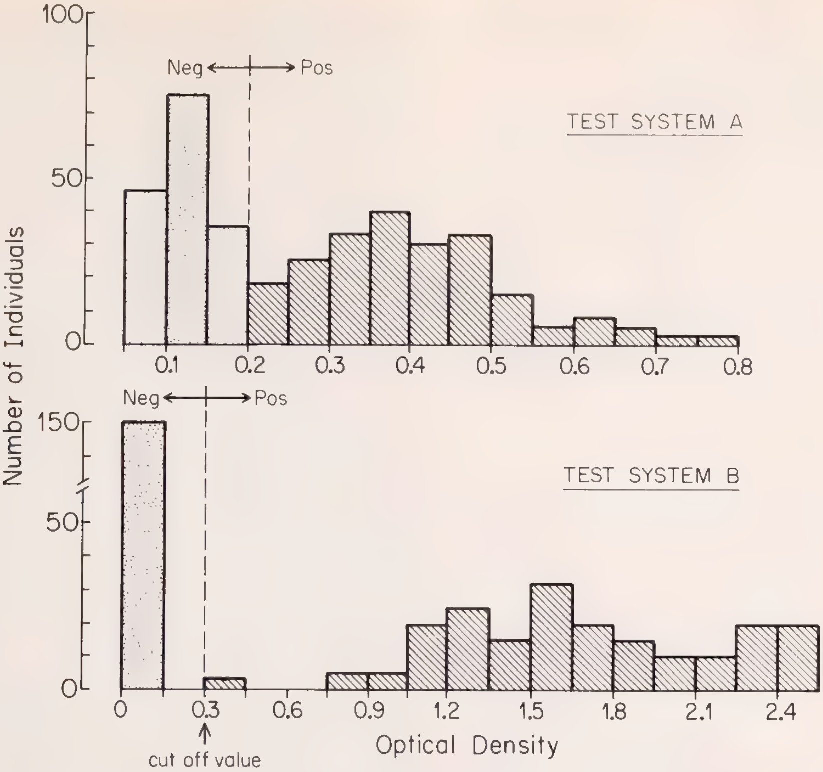

Fig. 2. ELISA tests for antibodies to HIV virus in 350 homosexually active men. Test system A: Litton Bionetics; test system B: Genetic Systems, Seattle, WA.

preconversion negative O.D. value (obtained within a six-month period before conversion) for this group was higher than the 50th percentile (Fig. 3). Also, the mean O.D. for the first positive test was below the 50th percentile, and the second positive test had a further increase following conversion, demonstrating that full antibody production following exposure is not always an immediate response but can stretch out over one year (Fig. 3).

To some extent, the number of such uncertainly "negative" persons will depend on the characteristics of the test system. A comparison of three commercially available ELISA test systems was made on a population of homosexual men at high risk for AIDS (6). The three commercial systems differed in source of antigen (LAV vs HTLV-III). Their results showed accuracies of 95%, 94%, and 85%, when compared with an immunoblot system. The system with the poorest correlations had a large number of results at or near the cut-off value.

These observations indicate that not all ELISA tests are equivalent, that quantitative testing by good ELISA methodology can be valuable, and that repeat and serial testing of some "negative" individuals are needed.

Evaluation of HIV-Caused Immune Deficits

Both immune depression and immune stimulation occur in response to the HIV infection. These two forms of immune response can persist through all the major stages of disease.

Immune Depression after HIV Infection. The major component of viral destruction within the immune system involves the CD4 cells, although reduced monocyte function and natural killer cell (NK) function may also be seen. It remains to be determined to what extent the reduced monocyte/macrophage function and NK reductions are secondary to changes in the CD4 population.

The CD4 (T helper/inducer) subset of T cells are directly infected by virus binding to the T4 molecule, which is a receptor for the HIV virus. Binding is followed in many cells by incorporation of virus into the cell, and in some cells, productive infection which can lead to cell lysis. In addition to direct lysis by HIV virus, the CD4 T

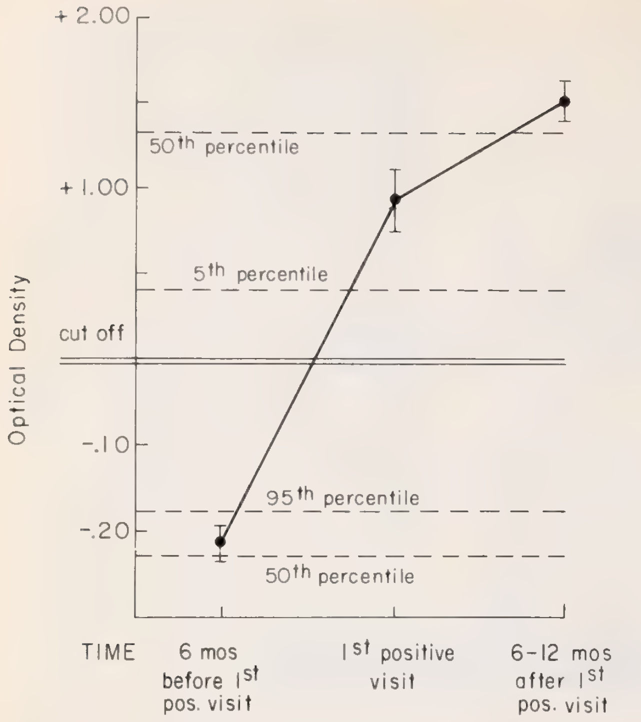

Fig. 3. Serial ELISA values for HIV antibody in 51 seroconverters. Mean negative optical density increases prior to seroconversion; mean positive value does not reach peak immediately on seroconversion.

cells are also impaired in function. Indeed, most T cells in the circulation do not have evidence of productive infection (9). Studies of the T helper/inducer population have revealed that the reduction generally involves all phenotypically identified subdivisions of this population (9). Apparently the T4 molecule is an effective binding molecule and can lead to infection in all of these subgroups of T cells. The reduction in CD4 cell number and depression of T helper/inducer cell functions are central to the pathogenesis of AIDS.

Immune Stimulation after HIV Infection. HIV also causes immune stimulation. This involves the CD8 T cells as well as the B cells. HIV infection is associated with increased serum immunoglobulins IgG and IgA, circulating immune complexes, increased autoantibodies, and increased spontaneous immunoglobulin secretion by B cells in the circulation. Furthermore, the virus has been shown in vitro to directly stimulate B cells to proliferation and immunoglobulin formation (10).

The CD8 (suppressor/cytotoxic) T cells also increase after HIV infection, and the increase generally persists throughout the illness. The principle component increased is one of the subpopulations of the CD8 cells, the Leu2+/Leu8− CD8 T cells (9), and other CD8 subgroups (11). These appear to be indicative of response to viral infection. Also, the DR and OKT10 antigens are increased in the CD8 T cells of AIDS patients (12). These later changes reflect T-cell stimulation and immaturity (13).

Some of the changes that are readily detected involve stimulation of the lymphoid system. Clinically, this is evident in the increased lymphadenopathy, often termed persistent generalized lymphadenopathy, that is a feature of AIDS-related complex. This response involves largely the CD8 T cells and the B cells (14). In the peripheral blood, the CD8 T cells are often increased, although the B cells rarely are. B-cell products, however, such as the immunoglobulins IgG and IgA (but usually not IgM), may be increased along with immune complexes. Other evidence of lymphoid stimulation can be seen in the increased serum beta-2 microglobulins, acid-labile alpha interferon and alpha-1 thymosin. In a study of AIDS patients with Kaposi's sarcoma (14, 15), IgG, IgA, and immune complex levels and CD8 T-cell measurements were specifically examined and found to be elevated frequently but not to correlate with prognosis.

Other Problems in Diagnosis and Prognosis

AIDS-Related Clinical Syndromes. AIDS-related complex (ARC) presents difficulties for clear definition in a clinical setting. This can be partly explained by the subjective nature of some of its symptoms (malaise, long-term lymphadenopathy) and differing opinions on which clinical, immunologic, and serologic parameters are to be considered (1, 16). If specific clinical parameters alone are considered (fever, weight loss, diarrhea, oral *Candida,* herpes zoster, night sweats, malaise), HIV-seronegative men who are at risk may be misclassified as ARC cases. If clinical definitions are too strict, they will not include HIV-positive individuals with vague but unmistakable systemic response to infection, such as neurologic deficit, or the recently described neonatal abnormalities in HIV-positive infants (17). As a result of this diversity of ARC definitions, analysis of prognostic factors in ARC has lagged behind similar analyses in AIDS-related Kaposi's sarcoma and opportunistic infections. Existing studies concur in demonstrating that reduced CD4 T-cell levels in ARC patients correlate with poor prognosis (18, 19).

Immunologic assessments in a group of 101

HIV-seropositive men with persistent generalized lymphadenopathy revealed a general correlation of CD4 T cell levels and CD4:CD8 ratio with progression to AIDS (18). The mean CD4 T cell subset was 178 ± 145 in those patients who progressed to AIDS, as opposed to 314 ± 195 in those who remained stable. Impaired gamma interferon has also been associated with progression from ARC to AIDS (19).

Neurologic Disease. Neurologic changes occurring in AIDS include manifestations of organic brain syndrome ranging from subtle recent memory loss to full-blown dementia (20). These are distinct from the psychological features associated with the stress and alienation associated with the diagnosis of AIDS or ARC (21). HIV and antibody to it can be isolated from the cerebrospinal fluid of most patients with neurologic symptoms (22, 23). AIDS virus has a capacity to infect the glial cells in the central nervous system, and the cellular changes are assumed to be responsible for organic brain damage.

Asymptomatic, HIV-Positive Persons. HIV virus affects the T-cell population directly, with both immune-cell damage and stimulation (24). In individuals who seroconverted over a known 6-month period, the majority showed a rise in CD8 T cells and a fall in CD4 T cells. The mean reduction in CD4 level within the first year was not as severe as the reduction seen in long-term (more than one year) seropositive persons (24, 25).

Some seropositive people do not progress to clinical disease, and some have milder forms. A relatively small proportion go on to advanced AIDS; these are the individuals who have the more rapid loss of CD4 cells. The likelihood of asymptomatic seropositive individuals developing AIDS relates to the CD4 cell level (and CD4:CD8 ratio). Data collected over an 18-month period from an ongoing study of 1,637 healthy homosexually active men (Table) show that when the CD4 level has fallen below 200 or the CD4:CD8 ratio is less than 0.2, the likelihood of AIDS is appreciable. A rough estimate is that within 3 years of initial infection, about 10%–25% of HIV-positive persons will develop significant immune depression, 5%–15% will develop ARC, and 2%–10% will develop AIDS (26). It is not certain, however, what percentage of persons who have acquired the HIV virus will be able to live a full life without some HIV-related disorder (27–29).

OKT10 Antigen Expression. Because significant disease usually appears after the HIV virus has caused considerable damage, a test indicating early but significant T-cell changes is needed. One of the immunologic changes that has been found to correlate with prognosis in AIDS-related Kaposi's sarcoma is the increase in number of cells expressing the OKT10 antigen (14, 15). This antigen is normally present on immature lymphocytes and also on activated lymphocytes (30). Expression of this antigen is frequently increased in AIDS (11, 12, 14).

The increase of OKT10 antigen is related to prognosis in AIDS patients with Kaposi's sarcoma, shown in the Kaplan-Meier plot of survival in a group of 97 such patients described elsewhere (15, 31) and in Fig. 4. These survival plots are similar to those seen with decreased CD4 T cell number and CD4:CD8 ratio in the same group. However, OKT10 increase is on the CD8 T cell subpopulation (13), and, presumably, reflects some other aspect of response to HIV infection.

TABLE

Initial CD4 T-Cell Level in Seropositive Men and Probability of Developing AIDS in 18 Months

CD4 Cell (#/mm^3)	Probability	CD4:CD8 Ratio	Probability
100	58%	0.1	69%
200	33%	0.2	36%
300	16%	0.4	13%
400	8%	0.6	6%
500	4%	0.8	3%

A portion of the Multicenter AIDS Cohort Study (MACS) funded by NIAID (Los Angeles, from 1984).

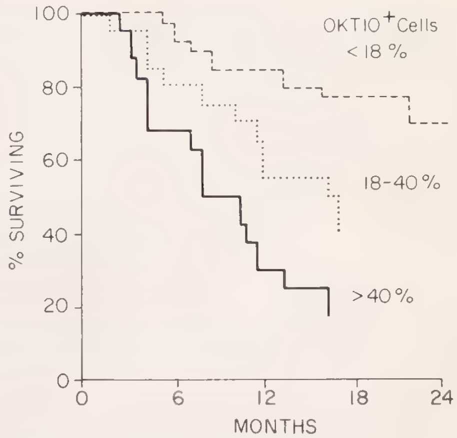

Fig. 4. AIDS patients with Kaposi's sarcoma: relationship of percentage of OKT10+ cells to survival. Measurements were made at time of diagnosis. Kaplan-Meier plot includes 29 patients with low OKT10 levels (<18%), 21 in the 18%–40% group, and 16 in the very high (>40%) group.

As noted earlier, HIV stimulates the immune system. Increased OKT10 antigen expression reflects the stimulation in the immune system by the virus. The amount of OKT10 antigen, furthermore, may be an index of the amount of HIV with access to the immune system. If this is the case, increased expression of the virus would be associated with further destruction of the CD4 cells. Thus elevated OKT10 expression would be an index of poor prognosis. Such is the case in AIDS-related Kaposi's sarcoma (Fig. 4). These observations are being extended to other populations with HIV infection.

Summary

The early detection of infection, using high-quality ELISA tests, and the monitoring of organ (lymphoid system) damage, using CD4 levels and CD4:CD8 ratio, are important features of the control of AIDS. Measurements of parameters such as OKT10 antigen and other tests being explored indicate that testing will be extended to include indications of active viral infection as well as of host resistance factors that contribute to containment of HIV infection and AIDS.

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Non-Hodgkin's Lymphoma in AIDS: Report of 11 Cases and Literature Review

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Abstract

Non-Hodgkin's lymphoma (NHL) has increasingly been linked to the acquired immune deficiency syndrome (AIDS) resulting from infection by human T-cell lymphotrophic virus type III, lymphadenopathy-associated virus (HTLV-III/LAV). These lymphomas tend to be of intermediate and high grade, involve extranodal sites, and are of B-cell origin. Primary central nervous system lymphoma has been associated with AIDS. Treatment of lymphoma in these patients is complicated by a high incidence of opportunistic infections and cytopenia. Non-Hodgkin's lymphoma in patients undergoing immunosuppressive therapy following organ transplantation may be an analogous situation, and reconstitution of the immune system is probably necessary for the adequate treatment of AIDS-associated lymphoma.

A virulent form of Kaposi's sarcoma and *Pneumocystis carinii* pneumonia (PCP), initially reported among homosexual men in 1981 (1), has been well described and is known to be associated with HTLV-III/LAV infection. In mid-1985 the case definition of AIDS was expanded by the Centers for Disease Control to include non-Hodgkin's lymphoma (NHL) of high-grade pathologic type and of B-cell or unknown immunologic phenotype, diagnosed by biopsy in the presence of a positive serologic or virologic test for HTLV-III/LAV (2). We report here a series of 11 patients with NHL associated with AIDS.

Materials and Methods

The charts of 11 adult patients with histologically proven NHL who had or were at risk for AIDS and were seen at Beth Israel Medical Center from January 1981 through December 1985 were reviewed. Serum antibody to HTLV-III/LAV, when it became available, was determined by an enzyme-linked immunosorbent assay (ELISA). Cytogenetic analysis including banding studies was performed on bone-marrow aspirates. All histologic material was classified according to the International Panel Working Formulation (3).

Results

Clinical and pathologic findings (Table 1) included an age range from 22 to 52 years. Homosexual males accounted for 9 of the 11 patients (82%); 2 patients, 1 a woman, were parenteral substance abusers. All but 1 had extranodal sites of disease, including bone marrow, chest wall, central nervous system, liver, and lungs. All NHLs exhibited a diffuse pattern. One homosexual man had an ulcerated anal mass with histologic components of large-cell lymphoma, immunoblastic type, and of Kaposi's sarcoma; a specimen of the mass grew herpes simplex virus. One parenteral substance abuser with leg weakness had a large psoas-muscle mass that was histologically a large-cell lymphoma.

Six patients had large-cell lymphoma, 2 of the immunoblastic type. Patient 5 was a male homosexual who had an initial diagnosis of an-

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TABLE 1

Clinical and Histologic Characteristics, 11 Patients with AIDS-Related Non-Hodgkin's Lymphoma

Pt. no.	Age	Risk group	Histology*	Stage	Extranodal sites
1	52	Homosexual	LCL; IB	IV	Liver, CSF
2	41	Homosexual	LCL; IB	II	Anus
3	28	Homosexual	LCL	IV	Primary CNS
4	37	Homosexual	LCL	IV	Bone marrow
5	42	Homosexual	AILD/LCL	IV	Lungs
6	33	IVDA	LCL	II	Psoas muscle
7	43	Homosexual	SNC	IV	Chest wall, bone marrow
8	22	IVDA (Female)	ML	II	None
9	29	Homosexual	LCL/Burkitt	IV	CSF, bone marrow, chest wall
10	41	Homosexual	Burkitt	IV	Disseminated
11	39	Homosexual	Burkitt	II	Chest wall

LCL, large-cell lymphoma; IB, immunoblastic; AILD, angioimmunoblastic lymphadenopathy; ML, mixed, small-large cell lymphoma; SNC, small, noncleaved; IVDA, intravenous-drug abuse.

* All histologic characteristics are diffuse type.

gioimmunoblastic lymphadenopathy (AILD) based on axillary node biopsy. Several months later, when he developed fevers, organomegaly, and increasing lymphadenopathy, a posterior cervical lymph node biopsy showed a focus of Kaposi's sarcoma in a lymph node replaced by AILD. When pulmonary infiltrates were noted on chest x ray, an open lung biopsy revealed large-cell lymphoma. Four of the six patients with large-cell lymphoma were stage IV initially; the others were stage II.

Three patients had Burkitt-type lymphoma. Two had stage IV disease; the third was stage II. Patient 9 initially had a large-cell lymphoma diagnosed by supraclavicular lymph node biopsy; a bone marrow biopsy was normal. Thirteen months later, repeat biopsy of the bone marrow showed Burkitt-type lymphoma (L3 morphology), cytogenetic analysis showing a t(8;14) translocation. Patient 10, with stage IV Burkitt-type lymphoma, also had reciprocal translocations involving the long arm of chromosomes 8 and 14. Autopsy of this patient confirmed disseminated lymphoma of the Burkitt type with brachial plexus involvement, compression of the larynx, gastric lymphoma, and lymphomatous infiltration of respiratory muscles. Cytogenetic studies of the third patient with Burkitt-type lymphoma were not performed.

Of the 2 remaining patients, 1 had a small, noncleaved cell (non-Burkitt type) lymphoma with bone-marrow involvement; the other had a mixed, small and large cell lymphoma, stage II.

All patients were treated with combination chemotherapy. Some required a change in regimen owing to refractoriness of the lymphoma. Those with, or believed to be at risk for, central nervous system involvement were treated with intrathecal chemotherapy and cranial irradiation. Nine of the eleven are evaluable for response to treatment and survival. Patient 11 was lost to follow-up, and patient 10 died within 24 hr of diagnosis, immediately following institution of chemotherapy.

Incidence of complications, treatment, response, and survival, Table 2: Five patients had PCP; in 2 this preceded the diagnosis of lymphoma. Two had herpes simplex virus infection, 1 prior to and the other following the diagnosis of lymphoma. Cytomegalovirus was cultured from the urine of 1 patient during an episode of unexplained fevers; in another it was cultured from oral mucosa. Two patients developed *Mycobacterium avium intracellulare* infection following the diagnosis of lymphoma. Two patients developed Kaposi's sarcoma after the diagnosis of lymphoma, and a third had Kaposi's sarcoma predating the lymphoma.

Three patients were under our care at the time HTLV-III antibody screening became available. All 3 were positive by ELISA (patients 1, 6, and 8). Four patients required multiple hospitalizations for leukopenia and sepsis related to chemotherapy. The 9 evaluable patients spent approximately 45% of their survival time in the hospital; range, 13%–100%. Median survival of evaluable patients is 7 months. Two patients are alive at 4.5 and 16 months.

Discussion

There is a substantial body of evidence that the immune system plays an important role in surveillance against the development of cancer (4). The risk of NHL is elevated in patients fol-

TABLE 2
Complications, Treatment, Response, and Survival, 11 Patients with AIDS-Related Non-Hodgkin's Lymphoma

Patient	Nadir sepsis	Opportunistic infections*	Kaposi's sarcoma	Treatment†	Response	Survival
1	Yes	PCP, CMV	No	PROMACE/MOPP IT MTX/RT	Complete	Dead 6 mo
2	Yes	HSV(P),PCP	Yes	CVP/RT	Complete	Dead 7 mo
3	No	PCP(P),HSV,CMV	Yes(P)	RT STEROIDS IT ARAC, MTX, DEC	Partial	Dead 2.5 mo
4	No	PCP(P),MAI	No	CHOP IT ARAC, HC, MTX	Partial	Dead 13 days
5	Yes	None	Yes	CHOP	Partial	Dead 11 mo
6	No	None	No	CHOP/MTX/RT	Partial	Alive 4.5 mo
7	No	None	No	PROMACE/MOPP	Partial	Dead 7 mo
8	Yes	PCP,MAI	No	CHOP/MTX	Complete	Alive 16 mo
9	No	None	No	CHOP-B/RT/M-BACOD APE/IT MTX	Partial	Dead 14 mo
10	—	None	No	CHOP	—	Dead 24 hrs
11	—	—	No	CHOP/MTX IT ARAC, MTX	Complete	Lost

* (P), prior to diagnoses of lymphoma; PCP, *Pneumocystis carinii* Pneumonia; HSV, herpes simplex virus; CMV, cytomegalovirus; MAI, *Mycobacterium avium intracellulare*.

† IT, intrathecal; RT, radiation therapy; HC, hydrocortisone; PROMACE-MOPP, prednisone, methotrexate with leucovorin rescue, doxorubicin, cyclophosphamide, etoposide, mechlorethamine, vincristine, prednisone, procarbazine; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; MTX, methotrexate; M-BACOD, methotrexate with leucovorin rescue, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone; CHOP-B, CHOP-bleomycin; ARAC, cytarabine; APE, cytarabine, cisplatin, etoposide; CVP, cyclophosphamide, vincristine, prednisone.

AUTHORS' NOTE: Patients 6 and 8 died one month after this paper was completed.

lowing organ transplantation with drug-induced immunodeficiency states (5). A significant proportion of these arise in unusual extranodal sites, including the central nervous system (6). Cyclosporin A is one example of such immunosuppressive therapy. T-cell-mediated immunity probably is suppressed by blocking an early stage in the activation of cytotoxic T lymphocytes in response to alloantigens (7). The ability of HTLV-III/LAV to produce immunodeficiency is now well known. This virus selectively infects the helper/inducer (T_4, Leu 3) T cell (8). The ability to suppress cell-mediated immunity shared by cyclosporin A and HTLV-III/LAV may explain the increased inci-

TABLE 3
Comparison of Four Series, Non-Hodgkin's Lymphoma in AIDS-Risk Groups

Series	Pathologic grade no. (%)*	Extranodal involvement no. (%)	CNS/CSF no. (%)	Median age
Ziegler et al (12)	H 56/90(62%) I 26/90(29%) L 6/90(2%) U 2/90(2%)	88/90(98%)	38/90(42%)	37
Ioachim et al (13)	H 4/18(22%) I 13/18(72%) L 1/18(6%)	8/18(44%)	4/18(22%)	37
Kalter et al (14)	H 0 I 13/14(93%) L 0 U 1/14(7%)	12/14(86%)	8/14(57%)	35
Levine et al (15)	H 22/27(81%) I 0 L 5/27(19%)	23/27(85%)	8/27(30%)	37
Present series	H 6/11(55%) I 5/11(45%) L 0	10/11(90%)	4/11(36%)	39

* H, high grade; I, intermediate grade; L, low grade; U, unclassified.

dence and unique properties of NHL in these two populations. Other clinical conditions associated with altered immunity, such as congenital immune deficiency, celiac disease, and sicca syndrome, all carry an increased risk of NHL (6).

Ziegler et al in 1982 reported 4 cases of Burkitt-like, undifferentiated, monoclonal, B-cell lymphomas in homosexual males from San Francisco (9). All had extranodal sites of involvement and died in less than a year. Chaganti et al (10) reported 2 male homosexuals with diffuse lymphomas of Burkitt type. Cytogenetic studies were performed, and abnormal karyotypes with reciprocal translocations characterized both patients. Both our patients with Burkitt-like lymphoma in whom cytogenetic studies were performed had abnormal karyotypes. Translocations of chromosomes 8;14 and 8;22 have been demonstrated in both the African endemic Burkitt's and nonendemic Burkitt's tumors from America, Europe, and Japan (11).

The incidence of extranodal sites of involvement as well as high-grade histologic findings and advanced-stage disease seems to be higher than expected in AIDS-associated NHL (12–15). The median age at diagnosis is 37, the age distribution of NHL in AIDS-risk male homosexual patients being almost identical to that seen in AIDS (12). Primary central nervous system lymphoma is a rare tumor (16). It often occurs in the presence of immunosuppression. Twenty-one of ninety male homosexuals with NHL were found to have primary lymphoma of the brain (12); this is an especially poor prognostic feature. Table 3 summarizes the clinical findings in four reported series of AIDS-related NHL and compares them to the series reported on here.

The development of NHL associated with HTLV-III/LAV infection portends a grave prognosis. Combination chemotherapy, although often effective in non-AIDS-risk patients, is often difficult to deliver adequately because of inherent myelosuppression and a very high rate of opportunistic infections. Chemotherapy and irradiation are of questionable value in the treatment of lymphomas in posttransplant patients immunosuppressed by cyclosporin A and steroids. Yet reduction or cessation of immunosuppressive therapy in these individuals often leads to complete remission of neoplasms, even when no other antitumor treatment is rendered (17). In these patients, enhancement of the immune system by decreasing doses of immunosuppressive agents was the only effective way to treat lymphoreticular malignancies. Treatment of AIDS-related NHL will probably continue to be disappointing until adequate immune replacement (or enhancement) and effective antiviral therapy are developed (18, 19).

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Lymphadenopathy in Patients at Risk for AIDS: Immunopathologic Study in Comparison to Controls

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Abstract

Histologic and immunopathologic findings in lymph nodes from 17 patients at known risk for the acquired immunodeficiency syndrome (AIDS) (male homosexuals and intravenous drug abusers) were compared to those from 9 patients not at known risk. The control lymph nodes were selected from a spectrum of lymphadenopathies, including unexplained follicular hyperplasia in non-AIDS-risk patients (3 cases), reactive hyperplasia to neoplasm or infection (4 cases), and unusual hyperplasias (1 case each, Dilantin-induced hyperplasia and X-linked lymphoproliferative disease). The helper-to-suppressor T-cell ratio, as determined by grid counting of immunoperoxidase monoclonal antibody stained preparations, was found to be abnormal in 16 of 17 lymph nodes from AIDS-risk-group patients (mean helper-to-suppressor ratio of 0.9). Fourteen of 17 patients demonstrated abnormalities of both follicular and paracortical T-cell subsets, 2 of follicular or paracortical only, and 1 was without detectable abnormality. In contrast, in controls, the helper-to-suppressor cell ratios were normal in 8 of 9 lymph nodes (mean helper-to-suppressor ratio 2.5 for paracortical T cells, 4.0 for follicular T cells). The single control case with abnormal helper-to-suppressor ratio was a lymph node from a child with persistent Epstein-Barr virus infection and X-linked lymphoproliferative disease, an immunodeficiency disorder. Evaluation of lymph node T-cell subsets by immunohistologic techniques may be useful in the differential diagnosis of AIDS-related lymphadenopathy.

Persistent generalized lymphadenopathy is an early manifestation of the acquired immunodeficiency syndrome related complex (AIDS-related complex) or ARC (1–3). In AIDS risk groups, persistent generalized lymphadenopathy is strongly associated with serologic evidence of infection by HTLV-III, the etiologic agent of AIDS (4), and may also be associated with the development of non-Hodgkin's (5, 6) or Hodgkin's (7) lymphoma or other manifestations of AIDS. The histopathologic finding in these cases of persistent generalized lymphadenopathy is non-specific follicular hyperplasia (1, 2). Studies have suggested that the lymph nodes may show immunologic alterations of T-lymphocyte subsets similar to those in peripheral blood (8, 9).

The development of lymphadenopathy in a patient at risk for AIDS is a significant problem (10). The histopathologic findings in AIDS-associated lymphadenopathy are striking but by no means specific to this syndrome (1, 2). Follicular hyperplasia, frequently unexplained, is a common reaction pattern in lymph nodes (11). Lymphadenopathy not associated with the AIDS complex may occur in homosexuals and intravenous drug abusers due to intercurrent illnesses, including pyogenic and viral infection and hepatitis. In the present study the applicability of immunologic marker studies to the study of lymphadenopathy in patients with risk factors for AIDS is examined. The immunopathologic findings in lymph nodes from 17 patients with known risk factors for AIDS are reported and compared with the findings in 9 patients without AIDS-associated risk factors representing a broad spectrum of reactive lymphadenopathies. Lymphadenop-

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athy in patients at risk for AIDS is commonly associated with immunopathologic alterations not found in most types of non-AIDS-associated lymphadenopathy.

Methods and Material

Patients. Patients were selected from those undergoing diagnostic lymph node biopsy. Patients were considered in the AIDS risk group if they met the criteria of (a) persistent generalized lymphadenopathy for more than 3 months, (b) known risk factors for the AIDS syndrome (male homosexuality or intravenous drug abuse), and (c) absence of criteria for the diagnosis of established AIDS (that is, opportunistic infection or Kaposi's sarcoma) at the time of lymph node biopsy. Prodromal manifestations associated with AIDS (unexplained fevers, weight loss, diarrhea) were present in some patients. Patients were considered in the non-AIDS-risk group if they did not belong to any group with known risk factors for AIDS and had either an identifiable cause for lymphadenopathy or unexplained lymphadenopathy in the absence of clinical features suggestive of AIDS. Peripheral blood lymphocyte studies were not used as a criterion.

Tissue. Lymph nodes were received fresh from the operating room. Tissue for routine histopathologic study was promptly fixed in 10% neutral formalin and processed for paraffin embedding. Tissue for immunopathologic studies was snap-frozen and stored in liquid nitrogen.

Immunopathology. Immunopathologic studies were performed on cold-acetone-fixed frozen sections. The three stage avidin-biotin-peroxidase complex technique was utilized with commercially available mouse monoclonal antibodies (Leu 1, Leu 2, Leu 3, IgG, IgM, kappa, lambda, Becton-Dickinson Inc.; T-11, Coulter, Inc.) (12). Immunopathologic preparations were counterstained with hematoxylin for cellular identification. T lymphocyte subsets were enumerated utilizing a 0.5 × 0.5 cm ocular grid at 450× magnification. Three representative fields were counted for each subset using standard grid counting techniques. Cases were counted by a single individual (RLP) without knowledge of the clinical history. Counts were expressed arbitrarily as cells per unit area without correction. Follicular and paracortical areas were evaluated separately. In some preparations, no follicles were identifiable and only paracortical areas were evaluated.

Results

Histopathology: Non-AIDS-risk group. Lymph nodes in the non-AIDS-risk group represented a broad spectrum of reactive lymphadenopathies (Table 1). Three lesions were follicular, five follicular and diffuse, and one entirely diffuse.

Histopathology: AIDS-risk group. Lymph nodes from patients in the AIDS risk group demonstrated a predominance of follicular hyperplasia (Table 2). Seven lesions were follicular, and ten follicular and diffuse. The pattern of follicular hyperplasia was marked with large ill-defined germinal centers. No purely diffuse lesions were encountered in this group, and no lymph nodes

TABLE 1

Control (Non-AIDS-Risk) Lymph Nodes

				Immunopathologic results									
				Follicles‡				Paracortex‡				Th:Fs/ratio	
Case	Age	Histo*	Diagnosis†	T-11	Leu-1	-2	-3	T-11	Leu-1	-2	-3	Follicles	Paracortex
1	18 F	Fo	Unexplained LA	32	26	12	41	67	77	31	60	3.4	1.9
2	45 M	Fo	Unexplained LA	—	16	5	21	—	56	29	57	4.2	2.0
3	71 M	F&D	Unexplained LA	—	49	16	40	—	105	25	127	2.5	5.1
4	26 M	Fo	Hodgkin's, reactive	—	11	3	12	–	36	21	44	4.0	2.1
5	57 F	F+D	Breast ca, reactive	—	8	12	24	91	76	16	73	2.0	4.6
6	88 F	F+D	Dermatopathic LA	32	33	6	28	48	50	16	41	4.7	2.6
7	2 F	F+D	Lung abscess, reactive node	32	—	2	20	180	—	54	82	10.0	1.5
8	78 M	D	Dilantin LA	—	—	—	—	—	62	29	51	—	1.8
9	6 M	F+D	X-linked lymphoproliferative	—	14	17	16	—	86	79	56	0.9	0.7
Mean				32	22.4	9.1	25.3	96.5	68.5	33.3	65.7	4.0	2.5

* Fo, follicular; F&D, follicular and diffuse; D, diffuse.

† LA, lymphadenopathy.

‡ Positive cells per 0.5 cm by 0.5 cm ocular grid area.

TABLE 2
AIDS-Risk-Group Lymph Nodes

					Immunopathologic results									
					Follicles‡				Paracortex‡				Th:Ts ratio	
Case	Age	Histo*	Risk	Symptoms†	T-11	Leu-1	-2	-3	T-11	Leu-1	-2	-3	Follicles	Paracortex
1	48 M	F	Gay	LA	20	14	16	11	83	75	71	71	0.7	1.0
2	43 M	F + D	Gay	LA	20	23	24	19	70	82	70	55	0.8	0.8
3	31 M	F	Gay	LA, fever, diarr	21	—	6	5	69	—	28	24	0.8	0.9
4	33 M	F + D	Gay	LA	—	56	36	11	—	100	95	44	0.3	0.5
5	39 M	F	IVDA	LA	18	18	12	27	65	64	58	70	2.2	1.2
6	56 M	F + D	Gay	LA	—	—	—	§	—	67	45	29	§	0.6
7	33 M	F + D	IVDA	LA, wt loss	—	—	—	§	29	35	21	26	§	1.2
8	27 M	F	Gay	LA	28	32	24	6	53	63	36	12	0.3	0.3
9	24 M	F + D	Gay	LA	—	31	21	16	—	82	47	38	0.8	0.8
10	42 M	F + D	Gay	LA	46	46	26	14	90	86	55	43	0.5	0.8
11	40 M	F + D	Gay	LA	14	24	20	11	48	54	38	28	0.6	0.7
12	29 F	F	IVDA	LA	—	17	21	14	—	65	61	38	0.7	0.6
13	30 M	F + D	Gay	LA, hepatitis	—	14	15	10	—	88	57	72	0.7	1.3
14	28 M	F + D	Gay	LA	—	21	23	20	—	81	71	43	0.9	0.6
15	28 M	F	Gay	LA	30	32	16	11	73	95	77	59	0.7	0.8
16	36 M	F + D	Gay	LA, hepatitis	14	—	8	22	71	—	23	41	2.8	1.8
17	36 M	F	Gay	LA, wt loss	22	49	25	32	104	79	60	105	1.3	1.8
Mean					23.3	28.7	19.5	15.3	68.6	75.1	53.7	46.9	0.9	0.9

* F, follicular; F + D, follicular and diffuse; D, diffuse.
† LA, generalized lymphadenopathy.
‡ Positive cells per 0.5 by 0.5 cm ocular grid area.
§ Follicles not evaluated.

demonstrating the depleted phase of established AIDS were encountered. A single lymph node demonstrated concurrent Kaposi's sarcoma not suspected prior to biopsy.

Immunopathology: Non-AIDS-risk group. All cases were polyclonal hyperplasias as evidenced by the presence of both kappa- and lambda-bearing B lymphocytes. Paracortical T-cell subsets were evaluated in 9 cases (Table 1). Total paracortical T-cell counts determined with Leu 1 yielded a mean of 68.5 (range 36–105). The mean helper-to-suppressor T-cell ratio (Leu 3/Leu 2) was 2.5. Eight of nine cases demonstrated a helper-to-suppressor cell ratio of 1.5 or greater. Follicle T-cell subsets were also determined in 8 cases. Total follicle T-cell counts with Leu 1 were lower than those found in the para cortex (mean, 22.4; range, 8–33). Helper-to-suppressor cell ratios in the follicles yielded a mean of 4.0. Seven of eight evaluable cases demonstrated a helper-to-suppressor cell ratio of 1.5 or greater in the follicles.

Immunopathology: AIDS-risk group. Paracortical T-cell subsets were determined in 17 cases (Table 2). Total T-cell counts with Leu 1 were similar to the non-AIDS-risk group (75.1, range 35–110). Helper-to-suppressor cell ratio, however was found to be decreased with a mean of 0.9. Fifteen of 17 cases demonstrated a helper-to-suppressor ratio of less than 1.5. Total follicular T cells were also similar to the non-AIDS-risk group (28.7; range, 14–52). However, total follicle suppressor cells were greater in the AIDS-risk group (19.5, range 6–36 versus 9.1, range 3–16). The helper-to-suppressor ratio in the follicles was decreased to a mean of 0.9. Thirteen of 15 cases demonstrated a helper-to-suppressor ratio less than 1.5.

Discussion

Persistent generalized lymphadenopathy in homosexual men has been associated with abnormalities of peripheral blood T-cell subsets, evidence of HTLV-III infection, and the subsequent development of AIDS in some patients (1–5). The histopathologic pattern of the lymph nodes in ARC is that of follicular hyperplasia (1, 2), a common reaction pattern in lymph nodes unassociated with AIDS (11). In the present study we applied an immunoperoxidase technique with mouse monoclonal antibodies to study the distribution of lymphocyte subsets in 17 cases of lymphadenopathy in patients belonging to AIDS risk groups (homosexual men and intravenous drug abusers) compared to 9 lymph nodes from

TABLE 3

T-Cell Subsets in Control (Non-AIDS-Risk) and AIDS-Risk Group Lymph Nodes

	Follicular T-cells		
	Cases evaluable	Leu 3/Leu 2 <1.5	Leu 3/Leu 2 1.5 or >
Non-AIDS-risk	8	1	7
AIDS-risk	15	13	2

($p < .01$ by chi square)

	Paracortical T-cells		
	Cases evaluable	Leu 3/Leu 2 <1.5	Leu 3/Leu 2 1.5 or >
Non-AIDS-risk	9	1	8
AIDS-risk	17	15	2

($p < .01$ by chi square)

non-AIDS-risk group patients, selected to represent a spectrum of lymphadenopathies encountered in hospital practice.

Sixteen of seventeen lymph nodes from patients in the AIDS risk group demonstrated immunopathologic abnormalities associated with ARC (Table 3). These included abnormal helper-to-suppressor ratios of paracortical or follicular T lymphocytes (mean Leu 3/Leu 2, 0.9) and increased follicular T suppressor cells. None of these patients had overt AIDS prior to biopsy.

In contrast, eight of nine lymph nodes from non-AIDS-risk patients demonstrated normal immunopathologic findings and normal helper to suppressor T-cell ratios (mean Leu 3/Leu 2 ratios of 2.5 and 4.0 for paracortical and follicular T cells respectively). These lymph nodes were selected from a spectrum of lymphadenopathies which might be considered in the differential diagnosis of AIDS associated lymphadenopathy, including unexplained follicular hyperplasia in non-AIDS-risk patients (3 cases), reactive hyperplasia to neoplasm (2 cases), bacterial infection (1 case), chronic dermatitis (1 case) and drug hypersensitivity (1 case). Of note, the only case in this group (case 9) which demonstrated immunopathologic abnormalities similar to the AIDS risk group was a lymph node from a child with persistent Epstein-Barr viral infection and familial X-linked lymphoproliferative disease, an immunodeficiency disorder.

Earlier studies have suggested abnormalities in lymph node T cells in patients with persistent generalized lymphadenopathy and ARC (8, 9). Two recent studies have used quantitative techniques to evaluate lymph node T-cell subsets in patients at risk for AIDS. Raphael et al (13) compared 12 lymph node biopsies from patients at high risk for AIDS to 7 conventional lymph node hyperplasias, utilizing an immunohistochemical technique and grid counting. They found reduced helper-to-suppressor cell ratios in the interfollicular, mantle zone, and germinal center area of lymph nodes from patients with generalized lymphadenopathy and ARC, in comparison to conventional lymph node hyperplasias. Of interest, five ARC patients were found to have normal peripheral blood T helper-to-suppressor cell ratios, suggesting the abnormality in the lymph nodes may precede that in the peripheral blood. Chan et al (14) studied 20 lymph nodes from homosexual men with generalized lymphadenopathy. The mean helper-to-suppressor cell ratio as determined by grid counting of sections and flow cytometry of cell suspensions was found to be significantly reduced. In contrast to Raphael et al, all of the patients also had abnormalities of the peripheral blood T helper to suppressor cell ratio, although correlation was poor. Unfortunately, in our study, the results of peripheral blood studies were not available for comparison. A prospective study, including the results of lymph node and peripheral blood T-cell subsets, would be desirable to determine the prognostic value of each in AIDS-risk patients.

Our study confirms the high incidence of T-cell subset abnormalities in lymph nodes from AIDS-risk patients. Fourteen patients demonstrated abnormalities of both follicular and paracortical T-cell subsets. Two other patients demonstrated abnormalities of either follicular or paracortical T-cells (cases 5 and 17) and one case demonstrated no abnormality (case 16). The case with no detectable abnormality may represent an early stage in the syndrome or alternatively may represent an intercurrent illness. Of note, the single patient in the AIDS risk group without immunopathologic abnormality had had recent viral hepatitis, which may be associated with generalized lymphadenopathy.

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Cytomegalovirus Endometritis in a Patient with AIDS

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Abstract

We report the case of a woman with irregular uterine bleeding, abdominal pain, fever, and weight loss for which no cause could be found. Her history of intravenous drug use, controlled by methadone, led to an analysis of lymphocytic markers, which showed an abnormal T-cell helper/suppressor ratio. A serologic test for HTLV-III/LAV antibodies was positive as well. Histologic examination of an endometrial biopsy showed cytomegalovirus (CMV) endometritis. This patient was at risk for exposure to the HTLV-III virus as a result of her intravenous drug abuse. Viral infection allowed CMV, an opportunistic infection in the compromised host, to disseminate and spread to the uterus, where the patient developed dysfunctional bleeding. This case, the first report of CMV in the female reproductive tract associated with acquired immune deficiency syndrome (AIDS), documents that irregular uterine bleeding may be a sign of CMV endometritis and of AIDS. With the increased incidence of AIDS in women, gynecologists should be alerted to this possibility. Further clinical studies are necessary to define the incidence, menstrual history, and optimum mode of therapy for this rare entity.

Cytomegalovirus (CMV) infection has been recognized for a long time. The histopathic effect of infection with this herpes virus produces a unique feature of cell enlargement and intranuclear inclusion. Until the 1960s, CMV infection was rarely identified, and then mostly in autopsies. In the acquired immune deficiency syndrome (AIDS) epidemic, CMV has become one of the most widespread and devastating opportunistic infections attendant on AIDS; along with *Pneumocystis carinii,* it is the cause of death by respiratory failure in a great number of immunocompromised patients.

Recently, cases of AIDS in women have become more numerous (1, 2). CMV in the female genital tract, however, has been reported in only a few cases predating the AIDS epidemic. Some of the reported cases were associated with abortions or with pregnancy and had devastating effects on the fetus (3–5). CMV oophoritis has also been reported (6).

Perhaps due to the relatively small number of women with AIDS and the rapid progression of the disease, CMV in the female reproductive tract has not previously been described in AIDS patients.

The purpose of this paper is to describe a case of CMV endometritis diagnosed in a patient with AIDS.

Case Report. The patient, a 28-year-old Hispanic woman, para 3002, came to the Emergency Room complaining of left upper quadrant abdominal pain that had developed suddenly six months before and had continued, accompanied by weight loss, sudden fevers to 101°F orally without chills or rigors and unrelated to the episodes of pain, and menometrorrhagia.

She had a history of intravenous drug abuse, maintained on methadone.

She also reported several years of amenorrhea, menstruation occurring every 3 to 4 months. For the three months prior to admission, however, she had bleeding 2–3 times per month.

On examination, the patient was noted to be an ill-appearing Hispanic woman in no distress. Her vital signs were stable; blood pressure was

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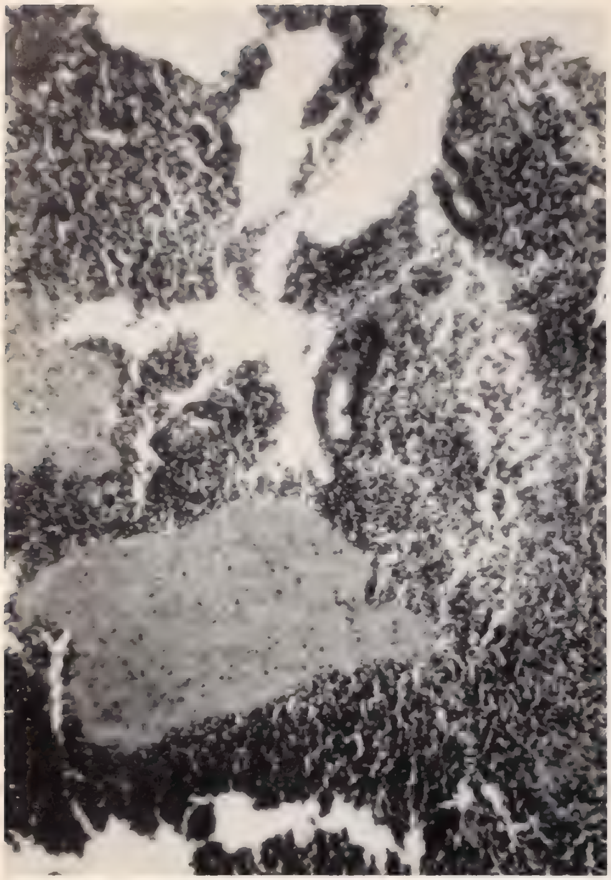

Fig. 1. Endometrial biopsy showing blood clots, necrotic tissue, and proliferative endometrium with numerous CMV intranuclear inclusions (hematoxylin and eosin ×50).

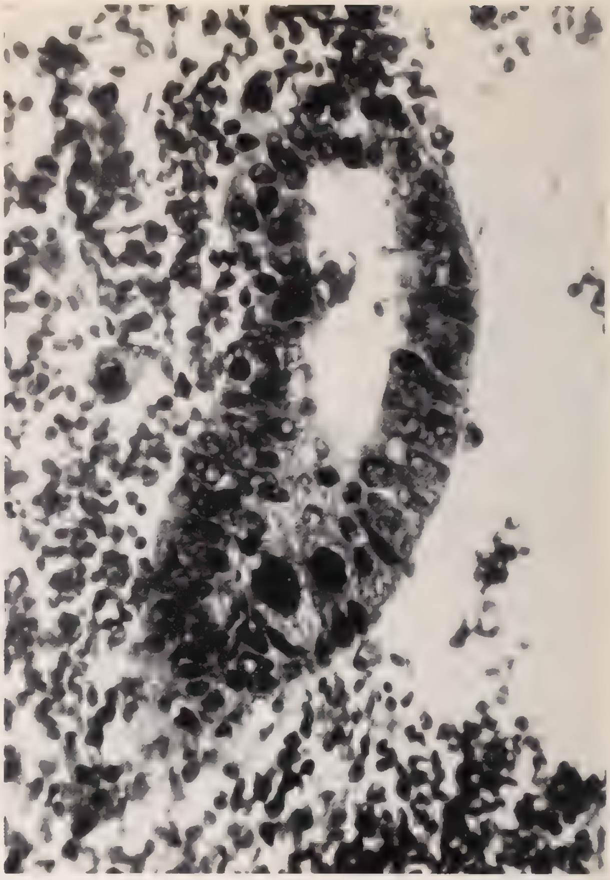

Fig. 2. CMV intranuclear inclusions affecting both glandular and stromal cells, which are enlarged up to ten times with large nuclei containing basophilic inclusions surrounded by clear halo separating it from nuclear membrane (hematoxylin and eosin × 200).

120/70 mm/Hg, pulse 75 bpm, respiratory rate 20/min, and oral temperature 98°F. Bilateral posterior cervical and axillary lymphadenopathy was noted. The nodes were 1–2 cm, firm, and nontender. Oral thrush was noted on her head and neck as well. Her chest was clear. Her abdomen was soft, and nontender except to deep left upper quadrant palpation. The uterus was firm and nontender and 10-12–week in size. Laboratory test values were as follows: hemoglobin 10.8 mg/dl, hematocrit 30.9%, white blood cell count 5.8 × 1000/mm^3, with 68% polymorphonuclear leukocytes, 5% bands, 5% lymphocytes, 18% monophils, 1% eosinophil; prothrombin time was 12/12. SMA 6 and 12 were normal, except for alkaline phosphatase, which was 151 ($n < 50$). Electrocardiogram and chest x-ray were negative.

The patient was admitted to the Medical Service for investigation of her left upper quadrant pain, fever, and weight loss.

Throughout hospitalization, her temperature was never elevated. The investigation was essentially negative except for the oral thrush and an enlarged uterus. In view of her history of intravenous drug abuse and oral thrush, an AIDS panel was performed. The patient's sera tested positive for HTLV-III (HIV) as measured by ELISA. Her T-cell helper/suppressor ratio was 0.8 ($n = 1.0–2.5$). These values indicate an abnormal T-cell helper/suppressor ratio consistent with AIDS.

Following a negative pregnancy test, an endometrial biopsy was obtained. Histologic examination of this tissue showed blood clots, necrotic endometrial tissue, and proliferative endometrium with numerous CMV intranuclear inclusions (Fig. 1). Numerous endometrial epithelial cells lining proliferative glands were markedly enlarged (up to 10 times the size of a normal endometrial epithelial cell); their nuclei were also enlarged and contained a basophilic inclusion, surrounded by a clear halo separating it from the nuclear membrane (Figs. 2, 3). Pap smear showed CMV-infected cells originating from the endometrium.

Further testing revealed a serum CMV titer

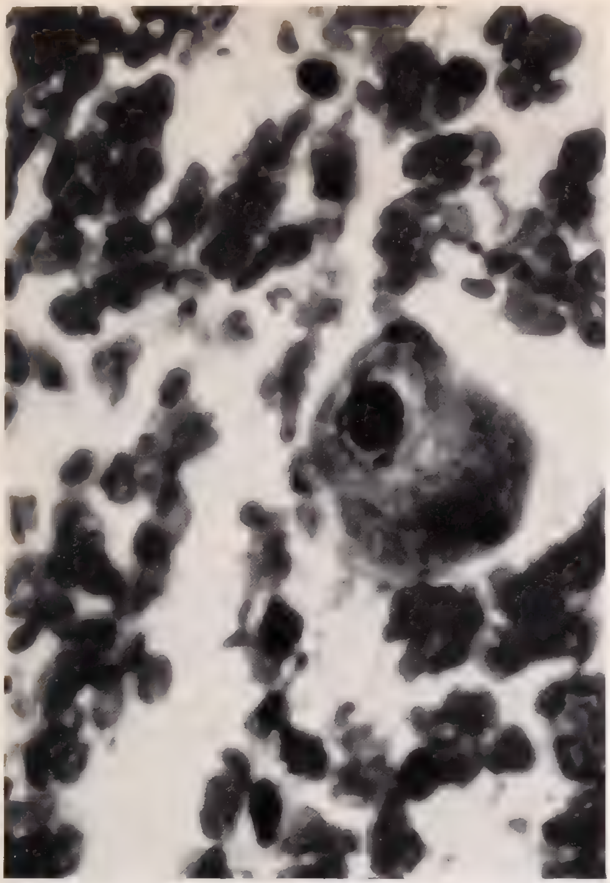

Fig. 3. High-power view demonstrating enlarged nucleus with basophilic inclusion surrounded by halo (hematoxylin and eosin ×600).

elevated to 1:256, as well as evidence of disseminated CMV documented on a bone-marrow aspirate.

Discussion. The morphologic features of the endometrium of this patient are characteristic of the cytopathic effect of CMV infection. Histologic changes are less sensitive diagnostically, but are nevertheless highly specific for the diagnosis of CMV infection; the histologic changes become apparent in highly virulent infections, and may be absent in weaker infections, detected by serum titers (7).

It appears that CMV tends to infect growing tissues (7), in this case the proliferative endometrium, in which normal mitoses were common. However, CMV endometritis is rare and reports of CMV endometritis in the literature are few. In 1973, McCracken et al. (8) reported the case of a 16-year-old girl with abdominal pain, vaginal bleeding, and fever enduring for 3–4 weeks who was diagnosed as having CMV endometritis.

In 1976, Wenckenback et al. (9) cites three case reports of genital tract CMV infection, only one of them involving the endometrium. In the other two cases, the only tissue affected was the cervix, a site reported on several earlier occasions in the literature (10, 11). CMV has also been successfully demonstrated to grow in vitro in human endometrial cells (12).

Opinions differ on whether the primary source of infection in the few reported cases of CMV endometritis has been by oral or sexual transmission. In the case we report here, the disseminated CMV infection was clearly secondary to the host's immunosuppressed state. The patient McCracken reported on had symptoms typical of a systemic CMV infection and similar to those of the patient we report on. However, in 1973 immunologic screening was in its infancy, so we can only speculate on that patient's immunologic status at the time of the report. With the prevalence of AIDS today and its increased occurrence in women, we believe the obstetrician and gynecologist must now be aware of the disease and the constellation of signs and symptoms associated with it.

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Cytomegalovirus-Induced Adrenal Insufficiency and Associated Renal Cell Carcinoma in AIDS

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Abstract

A 40-year-old man with the acquired immune deficiency syndrome (AIDS) and clinically documented cytomegalovirus infection had adrenal insufficiency, confirmed by an ACTH stimulation test. Extensive, necrotizing cytomegalovirus adrenalitis was demonstrated at autopsy. Renal cell carcinoma, a previously unreported finding in AIDS, was also present in this patient.

Several 1984 articles noted the occurrence of adrenal insufficiency, diagnosed by the ACTH stimulation test, in the acquired immune deficiency syndrome (AIDS) (1, 2). Others have reported the presence of cytomegalovirus adrenalitis at autopsy without an antemortem diagnosis of adrenal insufficiency (3, 4). We describe a patient with a clinical diagnosis of adrenal insufficiency, confirmed by an ACTH stimulation test, and autopsy-proven cytomegalovirus adrenalitis. He was also found to have a renal cell carcinoma, which, to our knowledge, has not been previously reported in AIDS patients, although several unusual malignancies, such as lymphomas, Kaposi's sarcoma (5), and, since 1985, small-cell carcinoma of the rectum (6) and metastatic small-cell carcinoma of the lung (7), have been well documented in AIDS.

Case Report

The patient was a 40-year-old bisexual, black male, who first came to The Mount Sinai Hospital with bilateral pneumonia and anterior and posterior cervical lymphadenopathy. He responded to a variety of antibiotics that included trimethoprim-sulfamethoxazole. Bronchoscopy with transbronchial biopsy was nondiagnostic.

The patient had one steady male sexual partner for many years. He inhaled cocaine, but did not use injectable drugs, and had never received blood transfusions. He had syphilis in 1979 and was seropositive for HB_sAg. Laboratory data were remarkable for anemia, leukopenia, diffusely elevated IgG levels, T helper–suppressor cell ratio of 0.38, and impaired pokeweed mitogen responsiveness.

A right renal mass was detected 1 month later and locally excised. The tumor was soft, tan-yellow, and measured 2.9 cm in greatest dimension. Microscopic examination revealed a moderately differentiated clear-cell carcinoma, grade II-III/IV nuclear atypia with infiltration of adjacent renal parenchyma, and a mild lymphocytic infiltrate (Fig. 1). The patient underwent a right radical nephrectomy 6 days later. No residual carcinoma was found in the resected kidney. Postoperatively he received antibiotics for low-grade fever and developed oral thrush, which was treated with oral ketoconazole.

Following discharge he gained 10 lb, but oral thrush persisted. He was treated with acyclovir for perianal herpes simplex infection and then

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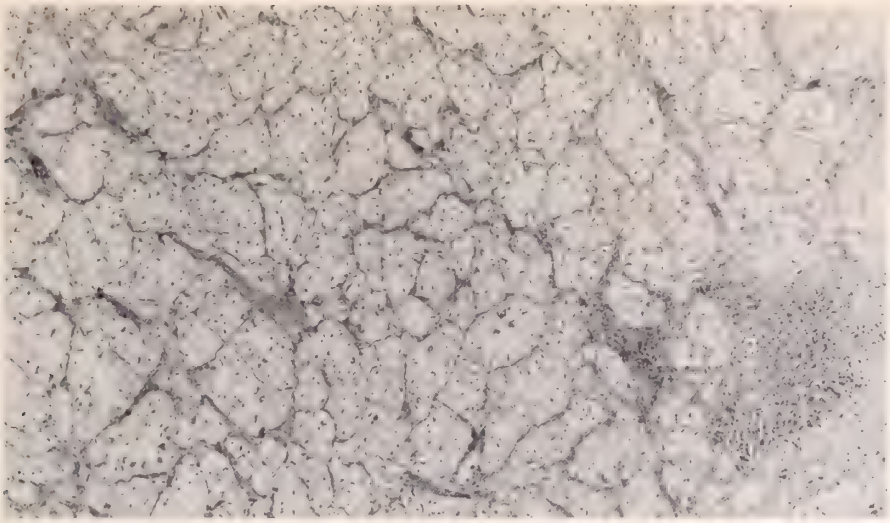

Fig. 1. Left Carcinoma of kidney composed of clear, vacuolated cells with small, round nuclei and mild lymphocytic infiltrate (hematoxylin-eosin, ×50).

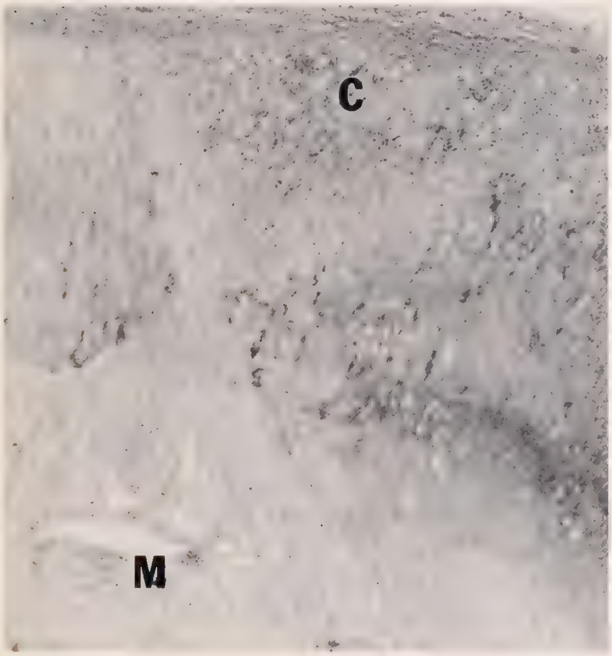

Fig. 2. Below, left Low-power photomicrograph of adrenal gland showing medulla (M) and wide areas of cortical necrosis (C) (hematoxylin-eosin, ×10).

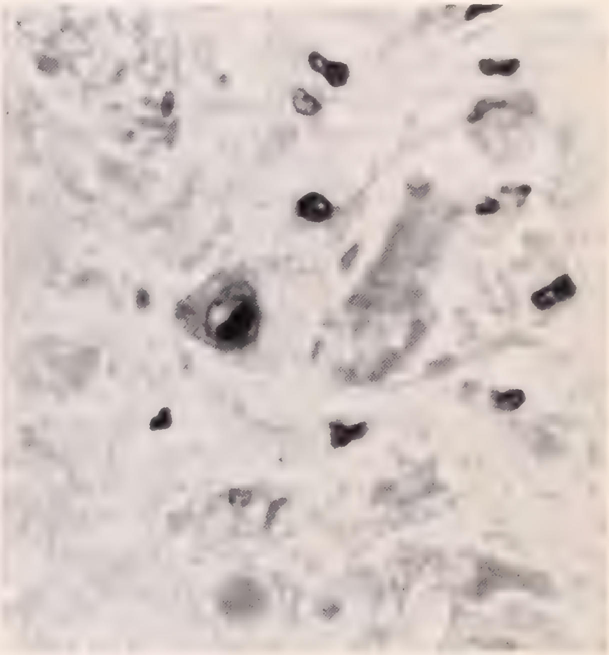

Fig. 3. Below, right Photomicrograph of adrenal gland revealing necrosis and a large cell with intranuclear and intracytoplasmic inclusions, characteristic of cytomegalovirus infection (hematoxylin-eosin, ×200).

developed generalized lymphadenopathy. Repeat immunological evaluation continued to exhibit a reversed T helper–suppressor cell ratio (0.40), and in addition to low pokeweed mitogen responsiveness, moderate impairment of phytohemagglutinin and concanavalin A responses.

The patient was readmitted 6 months later for recent onset of nausea, vomiting, and dehydration, as well as chronic bronchitis and recurrent oral thrush and perianal herpes simplex virus infection. Funduscopic changes consistent with cytomegalovirus infection were noted. He was depressed and had fallen several times. Neurologic examination was unremarkable. A computerized tomography scan of the head showed a questionable small, nonenhancing area of decreased attenuation in the left cerebellum. The patient responded to hydration and was discharged.

The patient was readmitted 1 month later, following a 1-week history of fever, worsening cough, progressive dyspnea, and dehydration. His temperature was 102°F, his blood pressure 80/50 mm Hg. Chest x ray showed a diffuse interstitial pneumonia. Serum sodium was 122 mEq/L, potassium 5.3 mEq/L, chloride 97 mEq/L, and CO_2 17 mEq/L. Arterial pH was 7.44, P_{CO_2} 23.4, and p_{O_2} 47 mm Hg on room air. Serum cortisol was 6.1 μg/dL (nl, 10–25), and there was no significant increase at 30 and 60 min following intramuscular administration of 25 units of ACTH gel. Bronchial lavage and transbronchial biopsy demonstrated *Pneumocystis carinii* and cytomegalovirus infection.

The patient was treated with intravenous saline, hydrocortisone, and trimethoprim-sulfamethoxazole. The sputum cultures from the previous admission grew *Mycobacterium avium intracellulare,* and isoniazid, ethambutol, rifampin, and clofazimine were added to the regimen. The patient initially defervesced and rapidly improved. On the twelfth hospital day, fever recurred, and the chest x ray showed progressive bilateral pneumonia. Pentamidine was substituted for trimethoprim-sulfamethoxazole. The patient continued to deteriorate and, despite intubation and respiratory support, died on the nineteenth hospital day.

Autopsy Findings. All lobes of the lung contained extensive *Pneumocystis carinii,* cytomegalovirus, and hemorrhagic necrotizing *Aspergillus fumigatus* pneumonia, as well as focal adult hyaline membrane disease. Disseminated cytomegalovirus infection was evidenced by numerous characteristic intracytoplasmic and intranuclear inclusion bodies in the stomach, colon, liver, spleen, and systemic blood vessels. Both adrenals were extensively necrotic and contained many CMV inclusion bodies accompanied by a mild to moderate lymphocytic infiltrate (Figs. 2, 3). Acid-fast bacilli were present in histiocyte aggregates in the liver, spleen, and lymph nodes. *Mycobacterium avium intracellulare* was cultured from the spleen. Perianal ulcers showed cytopathic changes diagnostic of herpes simplex virus. The spleen showed severe lymphoid depletion, especially in T-cell zones. The lymph nodes were devoid of follicles, lymphoid depleted, a few showing angiomatous proliferation. No evidence of Kaposi's sarcoma was found, nor was residual renal cell carcinoma seen. No lesions could be found in the brain.

Discussion

Numerous etiologies have been set forth for adrenal insufficiency since the syndrome was first described by Addison in 1855. In the past, tuberculous destruction of the glands was the most common cause in the United States; however, at present, the syndrome is commonly due to idiopathic or autoimmune adrenalitis (8). Other infectious causes include coccidioidomycosis, blastomycosis, histioplasmosis, and bacteria, especially meningococcemia (Waterhouse-Friedrichsen syndrome). One report also implicated adenovirus in an agammaglobulinemic patient (9). Several drugs have been associated with hypoadrenalism, notably corticosteroids, anticonvulsants (10), rifampin (11), and ketoconazole (12, 13).

With the mounting number of AIDS cases and the frequent autopsy finding of cytomegalovirus inclusion bodies within necrotic adrenal parenchyma (3), attention has been paid to the virus as a possible etiologic agent for adrenal insufficiency within the syndrome. In one study, CMV administered to T-cell-deficient mice consistently produced a weight-losing syndrome and CMV adrenalitis, whereas the control group of animals remained unaffected (14). Klein et al (15) studied 5 AIDS patients with signs and symptoms referable to hypoadrenalism, but ACTH testing revealed no adrenal insufficiency; no studies of CMV titers or other evidence of CMV infection were reported in these patients. Tapper's series (4) noted CMV adrenalitis in 8 of 10 autopsied patients, none of whom, however, had undergone clinical testing for hypoadrenalism. Primary Addison's disease with the subsequent development of AIDS was then reported (2). This patient had consistently low CMV titers, but did show CMV adrenalitis at autopsy. More recently, a 1984 report described 4 cases of clinically proven adrenal insufficiency and disseminated CMV infection (or the presence of anti-CMV antibodies), but autopsies were not permitted in these cases (1).

Our patient had a high CMV titer (+1/128) when he showed signs and symptoms of adrenal insufficiency confirmed by a positive ACTH stimulation test. CMV infection of the adrenals was associated with extensive necrosis. No other pathogen was detected to explain the adrenal insufficiency, although the long-term ketoconazole therapy may have contributed by blunting the cortisol response to ACTH stimulation (12, 13). Rifampin therapy could also have had a suppressive effect (11).

Although corticosteroids may have a therapeutic benefit in adrenal insufficiency associated with CMV adrenalitis in AIDS, a possible complication of such therapy is the increased risk of fatal *Aspergillus* infection (16), an organism not commonly detected in AIDS patients.

Unusual neoplasms have been associated with AIDS (5–7); two of them, immunoblastic B-cell lymphomas and Kaposi's sarcomas, are used as diagnostic criteria for the disease. This patient's clear-cell carcinoma of the kidney is of note because of its occurrence in association with AIDS as well as in a relatively young patient.

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Pulmonary Infiltrates in an Immunocompromised Patient

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Physicians in practice today are encountering the immunocompromised patient with increasing frequency. Many factors have contributed to the increased number of immunocompromised patients. These include the consequences of advancing age, drugs that cause neutropenia, myelosuppressive agents, and corticosteroid therapy. A host may be compromised as a result of environmental factors such as protein-calorie malnutrition, thermal injury, alcoholism, and irradiation. A major cause of this condition today is the acquired immune deficiency syndrome (AIDS) which has reached epidemic proportions in the United States in certain metropolitan areas. The acquired suppression of helper T-lymphocytes in patients with AIDS is the result of a retrovirus infection (HTLV-III/LAV). Pulmonary involvement is common. Numerous causes exist for pulmonary infiltrates in immunocompromised patients. More than one cause may be responsible for pulmonary infections. Almost any infectious agent can cause pulmonary disease in an immunocompromised host and pulmonary complications will result in death unless the clinician intercedes (1–9). The chest x-ray is never diagnostic of a single entity. We report yet another immunocompromised patient with bilateral pulmonary infiltrates.

Case Report. A 34-year-old man was admitted to the Bronx VA Hospital for evaluation of dry cough, dyspnea on exertion, and a 20-pound weight loss during the previous 6 months. He was an intravenous drug abuser for many years but was not homosexual or bisexual. One year prior to admission, the patient noted many nodular skin lesions and in August 1985 a biopsy of one of the lesions revealed Kaposi's sarcoma. A chest x-ray taken two years prior to admission was noted to be within normal limits. A dry cough was first noted three month prior to admission and at times produced clear sputum. There was no history of paroxysmal nocturnal dyspnea or orthopnea. He reported no known exposure to any toxin or tuberculosis.

On examination he was found to be well developed and in moderate distress; his temperature was 38.8°C, respiratory rate 18/minute, blood pressure 110/80 and heart rate 100/minute. His throat revealed extensive candidiasis; on the skin of his face, neck, trunk, and limbs were multiple purple subcutaneous nodules. He had diffuse 2 × 3 cm, firm, nontender mobile cervical, axillary, and inguinal adenopathy. Chest auscultation revealed bilateral respiratory wheezes in the upper lung field. An arterial blood gas on room air revealed a pH 7.41 and PO_2 57 mm Hg. The white blood cell count was 3,600/cu mm; hemoglobin, 13.8 gm/dL; platelet count, 142,000/cu mm. SMA-18 was unremarkable.

On admission, a chest x-ray (Fig.) demonstrated bilateral interstitial infiltrates. Treatment was started with trimethoprim sulfamethoxazole because of his history of drug abuse and a presumed diagnosis of *Pneumocystis carinii*. A fiberoptic bronchoscopy was performed the following day; a biopsy of the left upper lobe was obtained. Touch-preparation Giemsa stain and cultures for fungi and acid-fast bacterium were performed. After a 10-day course of trimethoprim sulfamethoxazole, the patient was discharged and was to be followed up in the chest clinic. Cul-

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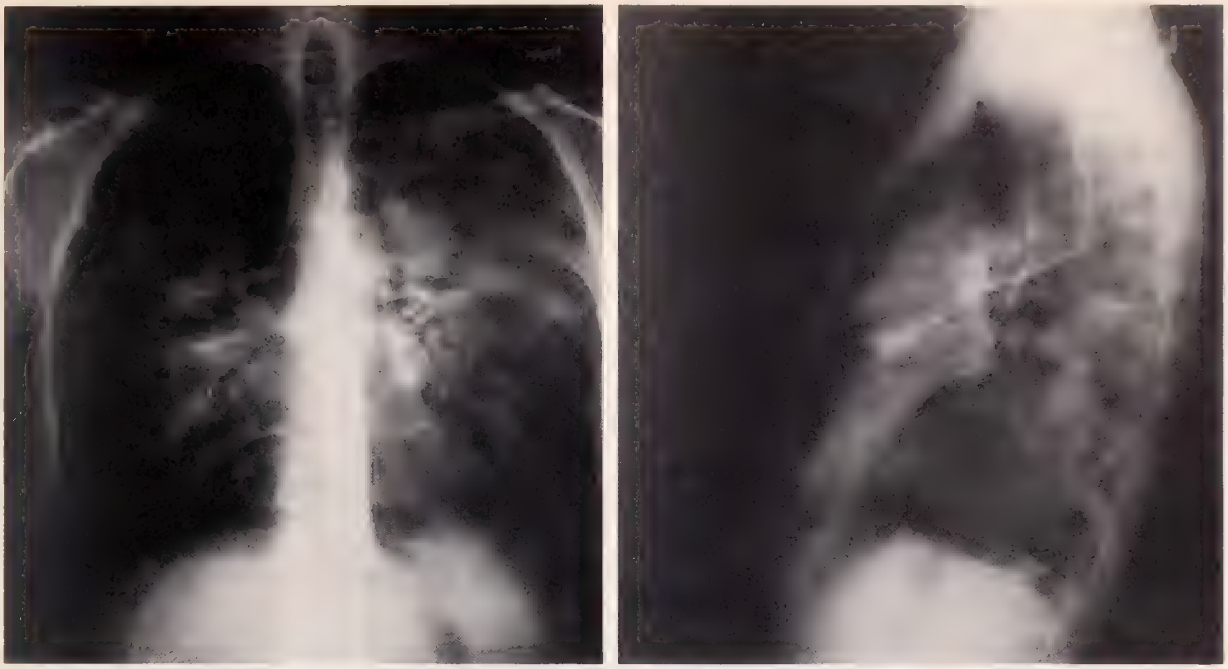

Fig. Chest x-ray in posteroanterior and lateral views showing bilateral pulmonary infiltrates.

tures of bronchoscopy specimens, stool, and urine were positive for atypical *Mycobacterium avium-intracellulare*. The pathology report on the transbronchial biopsy revealed Kaposi's sarcoma of the lung.

Discussion. The differential diagnosis of an interstitial pulmonary infiltrate in a febrile patient is extensive and includes bacterial pneumonias, *Pneumocystis carinii,* cytomegalovirus, atypical *Mycobacterium,* Kaposi's sarcoma, *Legionella,* hypersensitivity pneumonias, malignancy, and acute respiratory distress syndrome (1–9). The diagnosis may be further complicated if the individual is immunocompromised and has risk factors for AIDS. The diagnosis should not be based on clinical symptoms alone and is dependent on invasive tissue studies via open lung biopsy or bronchoscopy, including biopsy and lavage (1–3, 9). Pulmonary involvement is associated with high morbidity, and specific identification of the cause is necessary to assure adequate treatment (3, 7, 8).

This case illustrates several interesting caveats. The patient responded clinically to treatment for the presumed diagnosis, *Pneumocystis carinii* pneumonia, with intravenous trimethoprim sulfamethoxazole. He responded despite the presence of two additional lung diseases; tissue examination was positive for Kaposi's sarcoma and atypical *Mycobacterium avium-intracellulare*. Each of these diseases characteristically entails interstitial infiltrates (3, 6), and the combination raises new and yet unanswered questions as to therapeutic guidelines. In an immunocompromised host, tissue diagnosis is imperative. Multiple infections are the rule rather than the exception. One-third of these patients have two or more complications, including pneumonitis from two different opportunistic infections (3, 8). The lungs are involved in most complications and if lung disease is diffuse, the mortality approaches 50% (3, 8). Kaposi's sarcoma rarely originates in the lungs but can metastasize to that site. Nash et al in a recent autopsy study of 17 homosexual men found Kaposi's sarcoma in the lungs of 6 participants (6). Kaposi's sarcoma is an unusual finding in intravenous drug abusers who develop AIDS.

A cephalosporin, an aminoglycoside, antipseudomonal penicillin, and erythromycin is the recommended antibiotic regimen that can be instituted until a tissue diagnosis has been established (3). If the patient has a presumed diagnosis of AIDS, trimethoprim sulfamethoxazole alone or in combination with erythromycin can be used. In a seriously ill immunocompromised patient with pulmonary disease, the goal is to achieve a specific etiological diagnosis expediently and safely. Induced-sputum specimens are diagnostic 56% of the time for PCP and should be performed before bronchoscopy (10). If *Pneumocystis carinii* is not found on Giemsa stain, an adequate sample of lung tissue is needed for histologic examination, stains, and cultures so that adequate therapy may be instituted promptly (3, 8). Fiberoptic bronchoscopy with bronchoalveolar lavage and biopsy has been found to be useful in diagnosing pulmonary disease in patients with AIDS. The cost of empiric antibiotic therapy is appproximately 70% greater than that of bronchoscopy and bronchoalveolar lavage (3, 8). The risk-benefit and cost-effect of being aggressive with the immunocompromised patient with pulmonary infiltrates is justified. Furthermore, the potential toxic effects of empiric approach to therapy are avoided (11).

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Pulmonary Toxoplasmosis: An Unusual Nodular Radiographic Pattern in a Patient with AIDS

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Abstract

Following a long illness, a homosexual man with the acquired immune deficiency syndrome (AIDS) was admitted to the hospital with diffuse bilateral pulmonary infiltrates. Results of two transbronchial lung biopsies were nondiagnostic. The patient's condition worsened despite empiric therapy for *Pneumocystis carinii* pneumonia, and over several days a nodular density developed in the right upper lobe. The nodule was resected and revealed *Toxoplasma gondii* pneumonitis. This radiographic pattern has not previously been reported in pulmonary toxoplasmosis. This case illustrates the importance of histologic diagnosis in immunodeficient patients with pulmonary infection.

Toxoplasma gondii may be an opportunistic pulmonary pathogen in patients with the acquired immune deficiency syndrome (AIDS). The chest roentgenographic picture reported in immunocompromised patients with toxoplasmosis is diffuse bilateral pulmonary infiltration similar to that seen in *Pneumocystis carinii* infection (1). We describe pulmonary toxoplasmosis in a patient with AIDS with an unusual roentgenographic pattern: a large, single nodular density.

Case Report

A 33-year-old white homosexual man was admitted with dyspnea, night sweats, and intermittent fever of 3 months' duration. Stool examination several weeks prior to admission had shown cysts of *Entameba histolytica* and *Giardia lamblia*. The patient had received metronidazole and diiodohydroxyquin, with some improvement in the diarrhea.

Examination revealed a thin, ill-appearing man. Temperature was 102°F, heart rate 120/minute, respiratory rate 24/minute, and blood pressure 120/70 mm Hg. Examination of the head and neck revealed oral candidiasis and firm, nontender, mobile lymph nodes ranging in size from 5 mm to 10 mm in both occipital, anterior and posterior cervical regions, and both axillae. Chest examination revealed scattered rhonchi throughout both lungs. The heart, abdomen, genitalia, extremities, and nervous system were normal. Several skin lesions were noted, including a 2 × 3 cm tender, erythematous nodule over the left shoulder and several small purple lesions on the trunk and right thigh.

Initial laboratory data were hemoglobin 13.5 g/dl, hematocrit 40.7%, and white blood cell count 6400/mm^3 with 14% segmented neutrophils, 4% band forms, 48% lymphocytes, 5% monocytes, and 29% eosinophils. Arterial blood gas values on room air were pH 7.54, P_{CO_2} 27 mm Hg and P_{O_2} 80 mm Hg. The chest roentgenogram showed diffuse interstitial infiltrates bilaterally (Fig. 1). Because of the diffuse pulmonary infiltrates and

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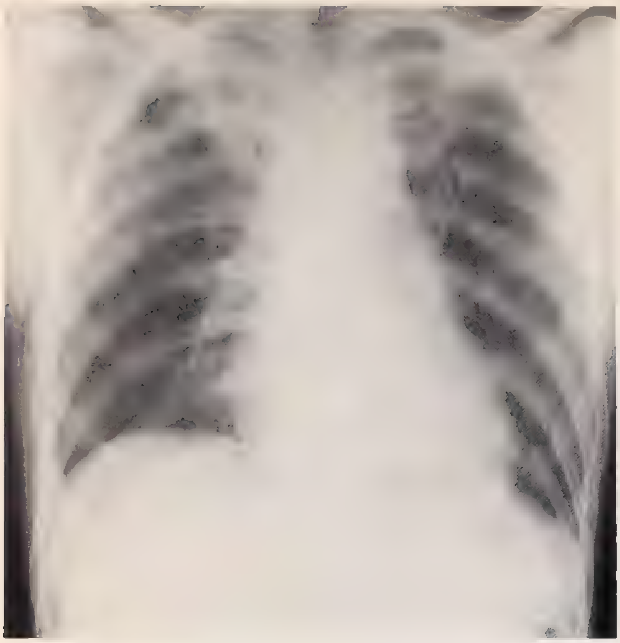

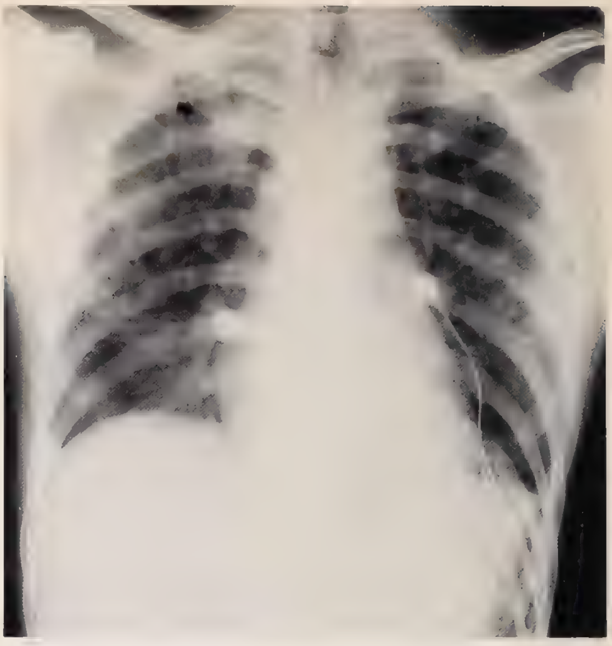

Fig. 1. Left Chest roentgenogram showing diffuse interstitial infiltrates bilaterally.
Fig. 2. Right Chest roentgenogram showing nodule in right upper lobe and diffuse interstitial infiltrates bilaterally.

the likelihood of AIDS, empiric therapy for *P. carinii* pneumonia with pentamidine isethionate was begun. Transbronchial lung biopsy performed shortly after admission, however, was normal. Biopsy of a right axillary lymph node and one of the skin lesions on the trunk revealed Kaposi's sarcoma, and biopsy of the left shoulder lesion revealed non-Hodgkin's lymphoma, large-cell type, with vascular involvement.

In view of these findings, doxorubicin (Adriamycin) and vinblastine treatments were begun. Repeat transbronchial lung biopsy was nondiagnostic. The diffuse interstitial infiltrates became more prominent over several days, despite continued therapy with pentamidine isethionate and, subsequently, cefazolin and tobramycin.

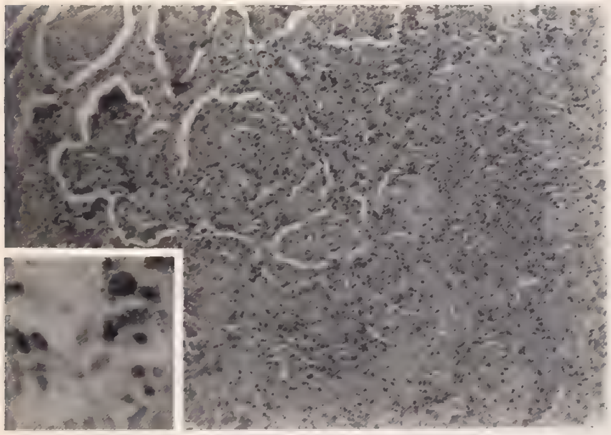

Fig. 3. Necrotizing *Toxoplasma* pneumonitis (hematoxylin-eosin, original magnification 40×). Inset: free *Toxoplasma* tachyzoites and eosinophils (Giemsa, original magnification 1000×).

On the tenth hospital day, a chest roentgenogram revealed a right-upper-lobe 2.5-cm nodule in addition to the diffuse infiltrates (Fig. 2). On the twelfth day, thoracotomy and open-lung biopsy were performed and the nodule was resected. Histologic examination revealed necrotizing *Toxoplasma* pneumonitis with marked eosinophilia (Fig. 3). Lung tissue adjoining the nodule showed no Kaposi's sarcoma, lymphoma, *P. carinii, T. gondii,* or cytomegalovirus pneumonia. Pentamidine and antibacterial therapy were discontinued and sulfadiazine and pyrimethamine treatments were begun for toxoplasmosis. Serum toxoplasma IgG antibody titer by the indirect fluorescent antibody technique was 1:16 during the first week of hospitalization and rose to 1:256 several weeks later. Following initial improvement in his respiratory status, the patient deteriorated steadily; he died 7 weeks after admission. Autopsy revealed severe cytomegalovirus pneumonia, and no evidence of Kaposi's sarcoma, lymphoma, *P. carinii,* or *T. gondii* in the lungs.

Discussion

Infection with *T. gondii* is common in the general population, and in the immunocompetent host causes asymptomatic lymphadenopathy (2). Occasionally, normal hosts develop chorioretinitis. *T. gondii* may cause more severe infection, however, in two distinct settings. First, when acquired in utero, it may lead to congenital toxoplasmosis with psychomotor or mental retardation, blindness, and intracranial calcification. Second, in patients with immunodeficiency, the infection may be serious, even life-threatening. A variety of clinical syndromes, most commonly diseases referable to the central nervous system, may be seen in immunodeficient patients.

In AIDS specifically, *T. gondii* is a common cause of intracranial mass lesions. It is not, however, a common pulmonary pathogen either in AIDS or in otherwise immunodeficient patients. A 1984 review of more than 400 patients with AIDS-related pulmonary disease included only one case of pulmonary toxoplasmosis (3). In a retrospective study of immunosuppressed patients with diffuse interstitial pneumonia from the National Cancer Institute and the National Institutes of Health, 1 of 22 patients had probable *T. gondii* infection (4).

Because of this low incidence, there are few descriptions of radiographic manifestations in pulmonary toxoplasmosis, and many published case reports lack histologic confirmation. In an early paper, Sante (5) divided the roentgenographic abnormalities of adult pulmonary toxoplasmosis in normal hosts into two stages. In the first stage, he described an increase in hilar shadows with accentuation of markings radiating outward into both lungs, similar to pulmonary edema. In the second stage, there is an irregular, blotchy consolidation of the lungs, involving mainly the lower lobes, similar to atypical pneumonias. The chest roentgenographic abnormalities in immunocompromised hosts have been described as diffuse interstitial and alveolar infiltrates, like the pattern of *P. carinii* pneumonia (1). Bilateral lower-lobe consolidation has been described in one patient with AIDS (6).

Our patient had a pulmonary nodule, which has not, to our knowledge, been previously described in pulmonary toxoplasmosis. The open-lung biopsy failed to reveal the cause of diffuse interstitial infiltrates seen on chest roentgenograms initially; however, we think these may have been due to cytomegalovirus infection, as the autopsy revealed only cytomegalovirus pneumonia.

This case reconfirms that roentgenographic patterns may be quite atypical in immunocompromised patients and that histologic diagnosis must be vigorously sought. Specifically, because of the difficulty that has been encountered with serologic studies in patients with AIDS, the diagnosis of invasive toxoplasmosis in these patients often cannot be made without tissue examination (7). This is especially true when infection occurs in an unusual site, such as the lung.

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Lymphoproliferative Disorder of the Central Nervous System in AIDS

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Primary lymphoproliferative disorders of the central nervous system are relatively infrequent. An increased incidence has been noted in patients with congenital immunocompromised states (for example, Wiskott-Aldrich syndrome) (1) as well as acquired immunodeficiencies, as in transplant recipients following immunosuppressive therapy (2–4). Since the recognition of the acquired immune deficiency syndrome (AIDS) in 1981, some affected patients have developed malignant lymphoma (5–12). A significant proportion of these tumors have involved the central nervous system, primarily or exclusively. The spectrum of lymphoproliferation in AIDS, which ranges from hyperplasia to lymphoma, may at times present difficulties in distinguishing a reactive from a neoplastic process. We report a patient with AIDS who developed a cerebral mass lesion which was characterized at postmortem examination as a primary lymphoproliferative process.

Case Report

A 37-year-old homosexual man was admitted to the hospital with a 2-month history of transient hemiparesis followed by a progressively deteriorating mental condition. He had several sudden attacks of hemiparesis and aphasia which cleared spontaneously within hours of onset. During and between the attacks, his mental status was observed to fluctuate and he became withdrawn and uncommunicative.

During the 6 months prior to this admission, he was treated for various opportunistic infections, including *Pneumocystis carinii* pneumonia, oral candidiasis, and anal herpes as well as Kaposi's sarcoma of the duodenum.

Examination revealed mild nuchal rigidity and an apical systolic murmur with tachycardia. The liver was palpable 14 cm. He had a low-grade fever of 101.6°F. Oral thrush and herpetic mucocutaneous lesions were noted in the perianal region.

Neurologic examination revealed that the patient was conscious but lethargic. There were cotton-wool spots in both optic fundi, with flame-shaped hemorrhagic discolorations. Occasional myoclonic jerks were observed in both lower extremities. Deep tendon reflexes were sluggish but symmetrical. Babinski signs were noted bilaterally. Muscle strength and pain and temperature senses were not impaired.

Significant laboratory findings included: BUN 35 mg %, alkaline phosphatase 177 IU, serum transaminases (SGOT 61 U/ml, SGPT 48 U/ml), amylase 98 U/ml. Creatine phosphokinase was elevated to 2,393 IU (normal, up to 150 IU). Gamma GTP was 267.

Cerebrospinal fluid was clear and colorless with normal opening pressure. It contained 11 white blood cells with 7 lymphocytes and 4 monocytes per high-power field. Glucose was 74 mg % and protein was 120 mg %. Testing for *Cryptococcus* antigen was negative, and the titer for *Toxoplasma* antigen was less than 1:2.

Serum antibody titers showed: Epstein-Barr virus 1:640, cytomegalovirus 1:16, herpes simplex 1:3. Hepatitis antigen screen test was negative; VDRL, ANA, and HBsAB were all negative. Blood culture did not grow organisms.

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Chest x rays showed increased interstitial markings in the perihilar regions. Computed axial tomography of the head showed normal configuration of intracranial structures. (This procedure was done 2 months before the final admission and was not repeated.)

Electroencephalogram showed diffuse bilateral slowing.

Course in Hospital. The patient was treated with sulfadiazine, pentamidine, and pyrimethamine, with the presumptive diagnosis of cerebral toxoplasmosis and chronic *Pneumocystis* pneumonia. A biopsy obtained from the left superior temporal gyrus did not yield a pathologic diagnosis. The low-grade fever persisted throughout the hospital stay and the patient's mental condition progressively deteriorated. He became comatose and died 4 weeks after admission.

Pathologic Findings. An autopsy was performed approximately 15 hr after death. The major findings included:

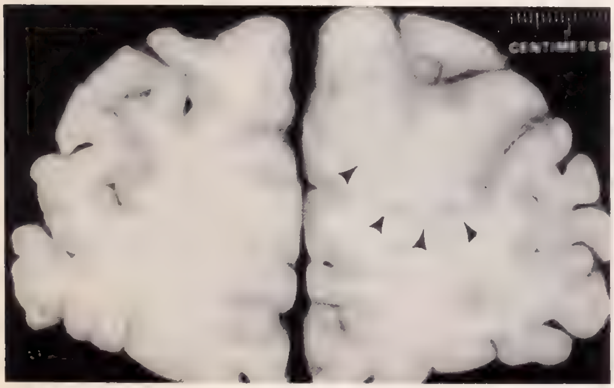
1. Disseminated cytomegalovirus infection involving lungs, spleen, duodenum, adrenal glands, and skin.
2. Mycobacterial infections of lymph nodes and spleen (*Mycobacterium avium-intracellularis*).
3. Kaposi's sarcoma of duodenum.
4. Bilateral bronchopneumonia with evidence of aspiration.

Examination of the nervous system revealed a moderately well-defined necrotic mass in the white matter of the frontal lobe on each side (Figs. 1, 2). One was situated in the central white matter of the right frontal lobe, the other in the left side of the genu of the corpus callosum. Microscopic examination showed an extensively necrotic lesion comprising numerous focal and confluent cellular aggregates of immature lymphoid elements. The cells had ovoid vesicular nuclei, often with a prominent nucleolus and minimal amphophilic cytoplasm (Figs. 3, 4). Some smaller cells were plasmacytoid in appearance. In addition to the large necrotic masses, multiple foci of lymphoid cell aggregates were disseminated throughout both hemispheres around many of the parenchymal blood vessels. The cells were found inside the vascular lamina, and they tended to aggregate around the perivascular spaces of Virchow-Robin. Histologic features suggested a lymphoproliferative process with characteristics of immunoblastic lymphoma. Immunoperoxidase reactions for cytoplasmic immunoglobulin, tested on formalin-fixed, paraffin-embedded sections, revealed IgG and IgA of kappa and lambda light chains, indicating a polyclonal B-cell-type proliferation.

Discussion

The primary brain tumors derived from the lymphoreticular system have been variously designated in the past as perithelial sarcoma, reticulum cell sarcoma, and microglioma. Recently, however, this group of intracranial neoplasms has been uniformly recognized as representing primary malignant lymphoma of the central nervous system and classified as non-Hodgkin's lymphoma (12–16). Most investigators have concluded that these lymphomas are predominantly of B-cell origin (17).

Frizzera et al (1, 2) have reported an increasing incidence of lymphoreticular disorders associated with primary and acquired immunodeficiencies. Others (3–11, 18) have confirmed this and added cases of acquired immune deficiency syndrome (AIDS). Many of these reports have included primary malignant lymphomas



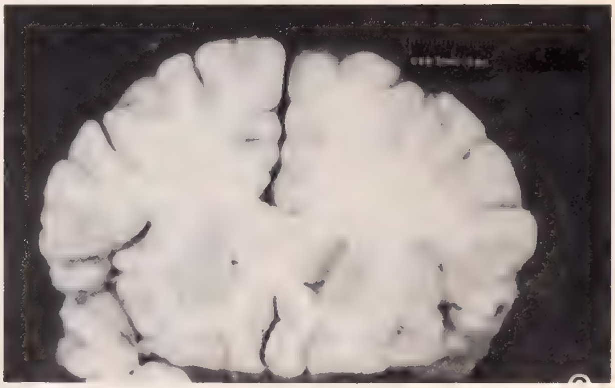
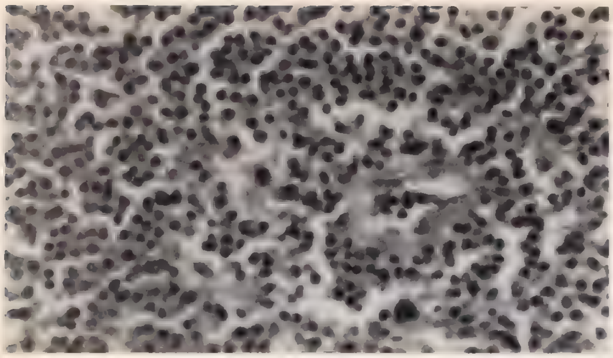


Fig. 1. Coronal section of brain showing a large, poorly defined mass in the right frontal lobe (arrows).
Fig. 2. Coronal section of brain showing swollen corpus callosum on left side.



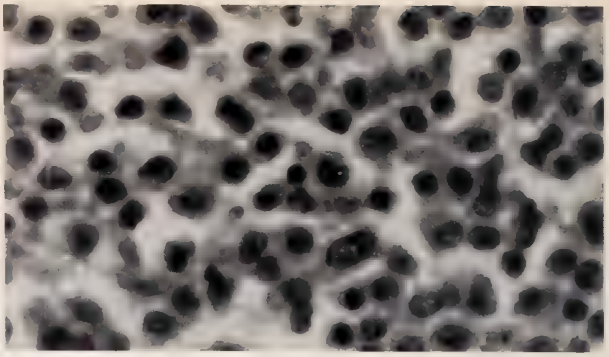


Fig. 3. Densely aggregated lymphoid cells (hematoxylin-eosin, ×700).
Fig. 4. Immature lymphoid cells with plasmacytoid features (hematoxylin-eosin, ×1,750).

confined to the central nervous system and made up of cells that consistently showed polyclonal reactions for cytoplasmic immunoglobulins (17, 18).

The negativity or polyclonality of immunoperoxidase reactions for cytoplasmic immunoglobulins in the cases of CNS lymphoproliferations in immunodeficient patients raises the question of the nature of this lymphoproliferative process—is it reactive or neoplastic (3, 4)?

Frizzera (2) proposed that if these lymphoproliferative disorders prove to be the expression of an abnormal immunologic response, rather than true neoplasia, new approaches should be directed toward correcting the immune dysfunction rather than killing the proliferating cells.

Reinforcing the concept of reactivity based on polyclonality, Hanto et al (4) and Hochberg et al (12) implicated Epstein-Barr virus in the pathogenesis of the lymphoproliferative disorders in immunosuppressed recipients of organ transplants, and recommended that these patients be treated with antiviral agents, such as acyclovir, at an early stage in the development of the lymphoproliferative process.

Cleary et al (3) reported monoclonality of the lymphoid cells in their series of cardiac transplant recipients, and concluded that the lesions may begin as a polyclonal proliferation which may progress to monoclonal disease, that is, true lymphoma. They proposed that antineoplastic treatment should be instituted at the earliest possible time. This group further justified their position by citing Hanto and associates' observation (4) that many of their renal transplant recipients with lymphoproliferative disorders developed resistance to acyclovir, coincidental with the appearance of a monoclonal population of proliferating lymphocytes.

Levine et al (19) found serologic evidence of antibody to human T-cell lymphotropic virus type III (HTLV-III) in 87% of homosexual men with high-grade lymphoma and proposed a potential etiologic relationship between the virus and malignant lymphoma. Their study, however, did not include lymphomas occurring in other forms of immune deficiency.

Since 1982, several complications have been noted to involve the central nervous system in patients with AIDS (8–11). These include subacute encephalitis, toxoplasmosis, cytomegalovirus infection, progressive multifocal leukoencephalopathy, cryptococcal meningitis, and malignant lymphoma.

The available diagnostic procedures are often suboptimal because of the rapidly progressive nature of AIDS and the multiplicity of infections that affect the central nervous system. Cerebral lesions found by imaging techniques, for example, computed tomography, require the differential diagnosis of infection versus lymphoma. Snider et al (9) and Pitchnik et al (20) presented relevant arguments concerning the merits of the biopsy of cerebral mass lesions.

In our patients, a homosexual man with frank AIDS for less than 1 year, neurologic symptoms developed during the final 3 months of his life. At autopsy, this proved to represent a lymphoproliferative process limited to the central nervous system. Brain biopsy was performed, but the specimen was inadequate. A mass lesion was located deep in the central white matter of the frontal lobe close to the genu of the corpus callosum. Since there was no meningeal involvement, the CSF cytology failed to show abnormal cells. Serologic antibody titer for Epstein-Barr virus was 1:640. Immunoperoxidase reactions for cytoplasmic immunoglobulins performed on the postmortem specimen showed IgG and IgA of kappa and lambda light chains, indicative of a polyclonal B-cell-type proliferation. This is considered a "prelymphoma" state by some authorities (3). Study of additional cases, perhaps with serial immunocytochemistry, may demonstrate the transformation of polyclonal to monoclonal cerebral lymphoproliferation in patients with AIDS.

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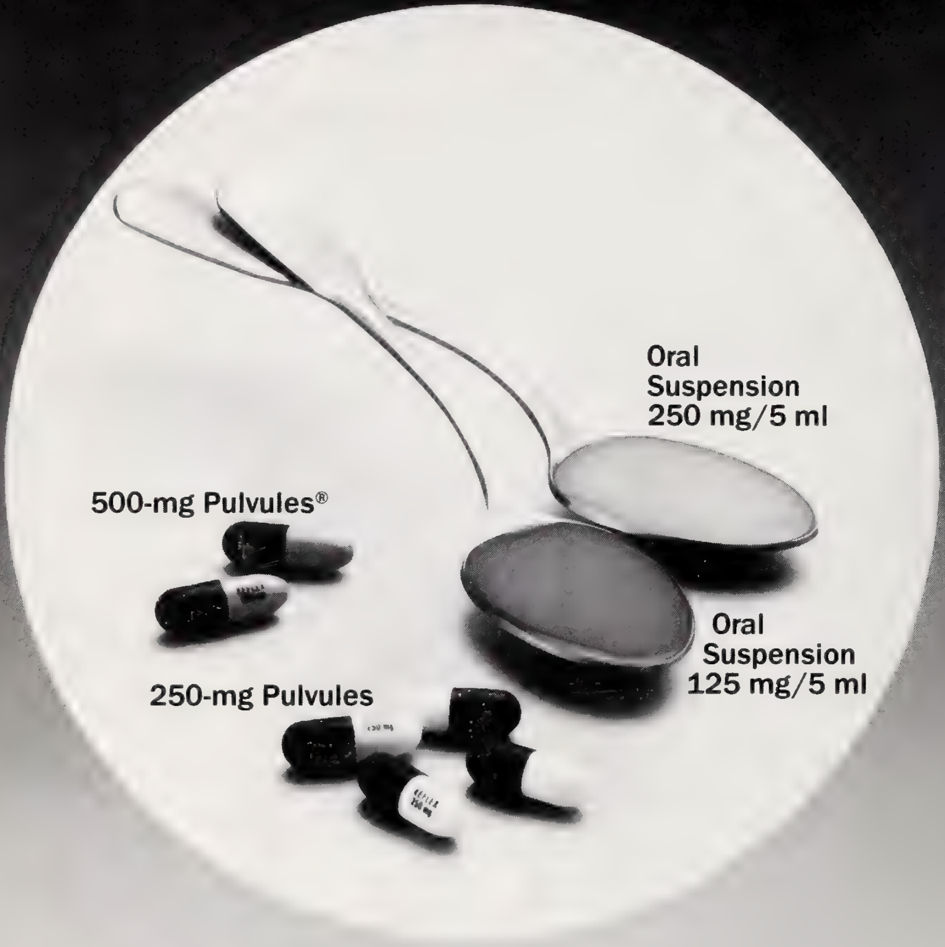
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